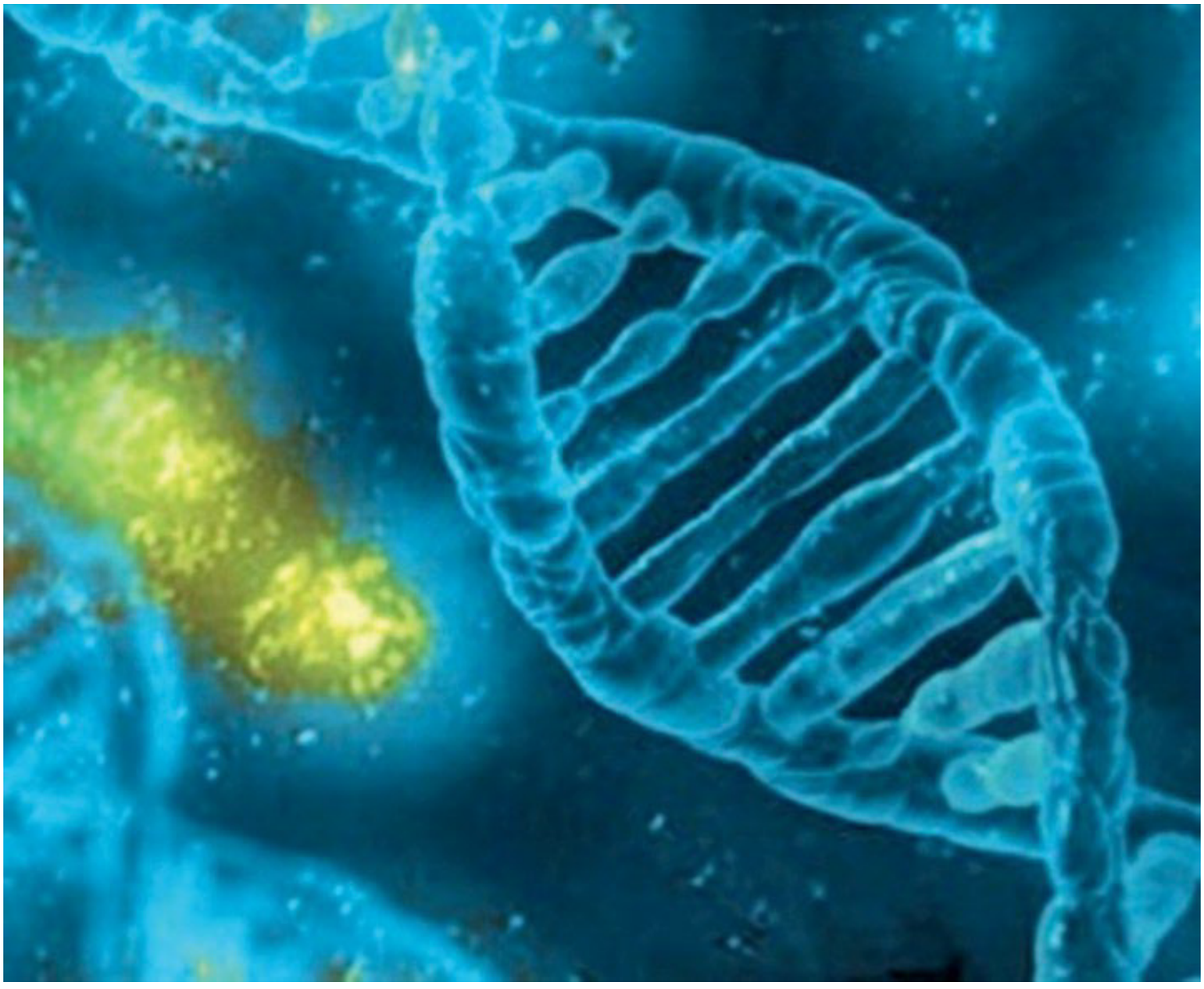
MOLTOX®

Molecular Toxicology, Inc.



Genetic Toxicology

MOLTOX®

Molecular Toxicology, Inc.

CELEBRATING

40 YEARS

SPECIALIZING in GENETIC TOXICOLOGY

Founded in 1986, MOLTOX® is a leading manufacturer of products used in mutagenicity tests. MOLTOX® minimal glucose agar plates, top agars, *Salmonella* and *E. coli* tester strains, frozen and lyophilized S9, MUTAZYME™, NADPH-regenerating systems, and positive control chemicals are distributed worldwide. MOLTOX® offers *Salmonella* and *E. coli* test kits in plate incorporation and fluctuation test formats.

MOLTOX® media are manufactured with the best materials:

Petri dishes are made from high-quality polystyrene, offering excellent optical clarity, dimensional uniformity, and stable stacking.

Many different containers, including PETG, PET, natural polypropylene, and polycarbonate containers with PTFE or white rubber closure liners, are used. All containers are noncontaminating.

Prepared media are made using industry-standard formulations and sourced from top-quality, reputable vendors.

Each lot of media is tested for sterility and its ability to support the growth of the appropriate microorganism, when applicable.

All media are accompanied by GLP-compliant formulation and QC certificates.

The MOLTOX® production facility is equipped with modern manufacturing equipment, including:

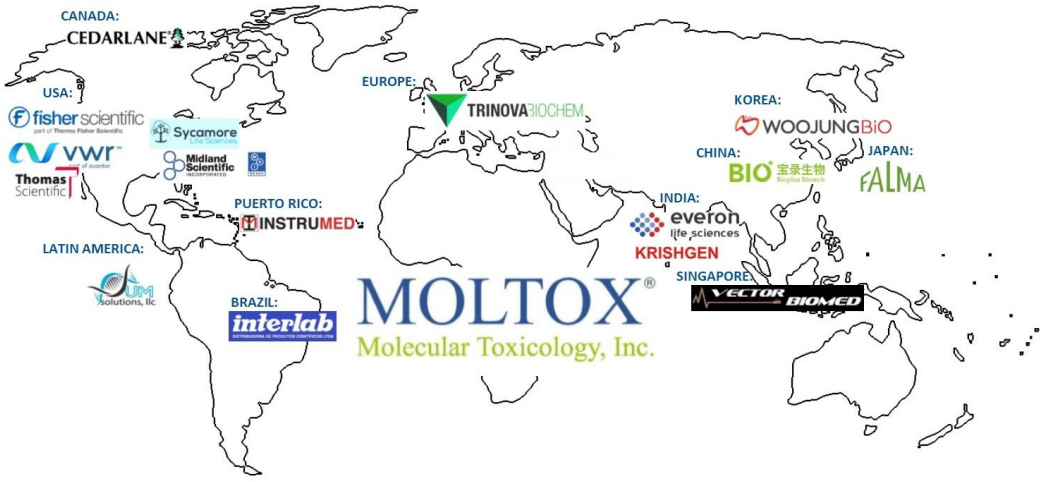
On-LineEngineering™ plate pouring machines

Automated media preparators

Automated bottle and tube filling assemblies

Get Social With Us!

DISTRIBUTORS



Canada

Cedarlane Labs
www.cedarlanelabs.com

China

Shanghai Bioplus Biotech Co., Ltd
www.bioplus-biotech.com

Europe

Trinova Biochem GmbH
www.trinova.de

India

Everon Life Sciences
www.everonlife.com

Krishgen Biosystems
www.krishgen.com

Japan

Falma
www.falma.co.jp

Latin America

Interlab Corp.
www.interlabdist.com.br

UM Solutions LLC
www.umsolutionsllc.com

USA

Fisher Scientific
www.fishersci.com

Midland Scientific
www.midlandsci.com

Sycamore Life Sciences
www.sycamorelifesciences.com

Thomas Scientific
www.thomassci.com

VWR Scientific
www.vwr.com

Puerto Rico

Instrumed Services Co., Inc.
www.instrumed.net

Singapore

Vector Biomed
www.vbm.com.sg

South Korea

Woo Jung BSC, Inc.
www.woojungbio.kr

MOLTOX®
Molecular Toxicology, Inc.

TERMS

Terms are Net 30 for approved account holders. Payment in USD only; drafts drawn against US banks. Please contact our office for wire transfer instructions. Expenses pursuant to international monetary transactions are the responsibility of the buyer. Additional shipping and processing charges may apply.

PRODUCTS

All MOLTOX® products are manufactured for research purposes only.
Please visit our website at www.MOLTOX.com for a full list of products.

ORDERING

Orders are processed, manufactured and shipped in the order they are received, based on raw material availability and scheduling requirements. Ship dates on Sales Order confirmations are estimates only. MOLTOX® reserves the right to change any ship date based on weather, raw materials, manufacturing conditions, storage conditions, etc., without penalty. Allow 24 hours for processing orders in the US and 48 hours for international orders. For more information, email sales@MOLTOX.com and for a list of distributors and website links, please visit www.MOLTOX.com

SHIPPING

Freight: Freight is Prepay & Add to invoice, CIF from Boone, NC. All orders requested to ship on collect accounts are FOB Boone, NC. For International Orders, additional packaging charges apply to dry ice and blue ice shipments. Shipping methods are based on transit time, storage conditions, and viability of the product. MOLTOX® reserves the option to change any shipment to 2nd day delivery or reconfigure standard packaging scenarios based on weather, storage temperature, transit time, etc., to ensure optimal delivery of goods. All dry ice shipments ship Overnight Priority.

Hazardous Goods: MOLTOX® is fully compliant with the packaging provisions set forth by sections 2.6.5 and 2.6.6 of the IATA Dangerous Goods Regulations and the US DOT CFR 173.4 & 173.4b of the Electronic Code of Federal Regulations. As a result, a Hazardous Goods Handling fee of $25 will be imposed on all International orders for those goods that fall under these regulations outlined in IATA 2.6 Dangerous Goods in Excepted Quantities.

Returns/Replacements/Credits: All made-to-order or non-restockable items are non-returnable. Any returns must be authorized by MOLTOX® and shipped at the expense of returnee. Freight damage must be reported within 7 days of receipt. All packaging must be kept for freight company inspection and photos of the packaging and damaged product must be obtained and sent to MOLTOX® for claims processing. Product functionality and/or quality issues must be made known to MOLTOX® as soon as possible for credit and/or replacement authorization. Products suspected or known to have been stored, used, or implemented improperly are not subject to replacement or credit.

STANDING ORDERS

MOLTOX® encourages standing orders. Please contact our customer service department for more information at sales@MOLTOX.com

TO ORDER:

CALL:

Monday - Thursday
8:30am – 5:00pm (EST)
US/Canada: 828.264.9099 or
800.536.7232
INTL: 001.828.264.9099

EMAIL:

SALES: sales@MOLTOX.com

CUSTOMER SERVICE:

sales@MOLTOX.com

www.MOLTOX.com

MOLTOX®
Molecular Toxicology, Inc.

157 Industrial Park Dr.
Boone, NC 28607 USA
Mailing Address
PO Box 1189
Boone, NC 28607 USA

S9

S9 preparations and cofactor reagents for use in metabolic activation studies are manufactured to rigorous standards of quality and performance and are used by leading research, government, and academic laboratories worldwide.
S9 and associated cofactors supply metabolic activation otherwise not present in genetic toxicology test systems.
Use in any assay that requires an exogenous source of metabolic activation.

Frozen S9 preparations require ultra-low storage conditions, ideally at -80°C.
This type of S9 requires expedited shipping on dry ice and careful temperature considerations during the thaw process.
Once thawed, your S9 is ready for use with cofactors.

All PB/BNF induced S9, including MUTAZYME™ has undergone the induction treatment as described per Matsushima et al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

For more information, please refer to pages 54 -56.

FROZEN S9 (-80°C)

Phenobarbital/5,6-Benzoflavone

Sprague Dawley

Post mitochondrial supernatant prepared from Sprague Dawley male rat liver. Prepared using the treatment schedule of Matsushima, et. al. 1976. In: de Serres, F.J, et, al., ed. In Vitro Metabolic Activation in Mutagenesis Testing. Amsterdam (NL) Elsevier/North Holland p. 85-88.

11-105.1	1 ml	1 each	**Frozen**	Male Rat liver in 0.15 M KCl	Ultra Low (-80°C)
11-105.2	2 ml	1 each	**Frozen**	Male Rat liver in 0.15 M KCl	Ultra Low (-80°C)
11-105.5	5 ml	1 each	**Frozen**	Male Rat liver in 0.15 M KCl	Ultra Low (-80°C)

Uninduced

Sprague Dawley

Post mitochondrial supernatant prepared from untreated Sprague Dawley male rat liver. For use in control experiments.

11-102.2	2 ml	1 each	**Frozen**	Male Rat liver in 0.15 M KCl	Ultra Low (-80°C)
11-102.5	5 ml	1 each	**Frozen**	Male Rat liver in 0.15 M KCl	Ultra Low (-80°C)

Phenobarbital/5,6-Benzoflavone

Golden Syrian Hamster

Post mitochondrial supernatant specifically prepared for use in the Enhanced Ames Test (EAT). Also suitable for use in the "Modified" Ames test for petroleum oils.

15-205.1	1 ml	1 each	**Frozen**	Male Hamster liver in 0.15 M KCl	Ultra Low (-80°C)
15-205.5	5 ml	1 each	**Frozen**	Male Hamster liver in 0.15 M KCl	Ultra Low (-80°C)

Uninduced

Golden Syrian Hamster

For use in control experiments or for application in the "reductive" protocol as described by Prival & Mitchell, Mutat. Res. 97:103, 1982.

15-104.5	5 ml	1 each	**Frozen**	Male Hamster liver in 0.15 M KCl	Ultra Low (-80°C)

LYOPHILIZED S9 & MUTAZYME™

Freeze-dried using a proprietary process that confers exceptional stability.
Especially useful where an ultra-low freezer is not available and where dry ice shipments are difficult or prohibitively expensive.
Can be stored in an ordinary –20°C freezer where an ultra-low is not required.
Ready for use after reconstitution with the labeled volume of cold, sterile, purified water.

Phenobarbital/5,6-Benzoflavone

Sprague Dawley

Post mitochondrial supernatant prepared from Sprague Dawley male rat liver. Prepared using the treatment schedule of Matsushima, et. al. 1976. In: de Serres, F.J, et. al., ed. In Vitro Metabolic Activation in Mutagenesis Testing. Amsterdam (NL) Elsevier/North Holland p. 85-88.

11-05L.1	1 ml	1 each	**Lyophilized**	Male Rat liver in 0.15 M KCl	Freezer
11-05L.2	2 ml	1 each	**Lyophilized**	Male Rat liver in 0.15 M KCl	Freezer
11-05L.5	5 ml	1 each	**Lyophilized**	Male Rat liver in 0.15 M KCl	Freezer

Phenobarbital/5,6-Benzoflavone

Golden Syrian Hamster

Post mitochondrial supernatant specifically prepared for use in the Enhanced Ames Test (EAT).
Also suitable for use in the "Modified" Ames test for petroleum oils.

15-05L.5	5 ml	1 each	**Lyophilized**	Male Hamster liver in 0.15 M KCl	Freezer

MUTAZYME™ is a lyophilized S9 mix, complete and ready for use after reconstitution with cold sterile purified water.

No secondary reagents/solutions required

- Can be stored in an ordinary –20°C freezer where an ultra-low is not required.
- Completely characterized and contains the S9 and NADPH regenerating system recommended by Mortelmans and Zeiger (Mortelmans, K. and E. Zeiger, The Ames Salmonella/microsome mutagenicity assay. Mutat. Res., 455: 29-60, 2000).
- Recommended for use in Chromosome Aberration and In Vitro Micronucleus Assays.

MUTAZYME™ consists of PB/BNF induced male SD rat liver S9 Mix.
After reconstitution, the resultant S9 Mix is ready for use.

Offerings include 5% and 10% S9 concentrations for use in the Ames Assay (standard, micro, pre-incubation) and 30% for use in microtiter fluctuation tests (e.g., MOLTOX® FT™ tests).

MUTAZYME™ products are subjected to the same Quality Control analyses as MOLTOX® frozen and lyophilized S9.

Each lot is accompanied by a C of A which include the results of bioassay and biochemical analyses such as alkoxyresorufin-0-dealkylase activities, total protein content, S9 titrations vs. treatments of TA100 with B(a)P and 2-AA, and TA1535 and TA98 responses to CP and EtBr, respectively.

Lyophilized MUTAZYME™

11-404L	20 ml	1 each	**Lyophilized**	10% S9 Mix, PB/PNF Induced	Freezer
11-405L	20 ml	1 each	**Lyophilized**	5% S9 Mix, PB/PNF Induced	Freezer
11-406.3L	3.25ml	1 each	**Lyophilized**	30% S9 Mix, PB/PNF Induced	Freezer

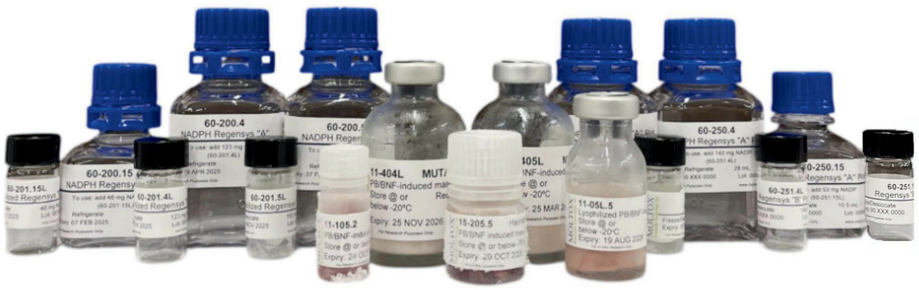
Regenerating Systems

Convenient preformulated, filter-sterilized, and prepackaged S9 mix components for the preparation of
4 - 10% S9 mix Regensys™
&
Regensys™ Plus is formulated for the preparation of 5 - 30% S9 mix.
Ideal for use in the Enhanced Ames Test.

Regenerating Systems are needed to activate the S9.
The MOLTOX® Regensys™ product line meets this need.
Regensys™ "A" contains KCl/MgCl, Sodium Phosphate Buffer (0.2 M, pH 7.4), and Glucose-6-Phosphate.

Regensys™ "B" is lyophilized (i.e. freeze-dried) NADP.
One can add the Regensys™ "B" and S9 (and sterile, cold, purified water if applicable) directly to the Regensys™ "A" bottle, for ease of use.

See pages 57- 60 for concentration tables.



REGENERATING SYSTEM REAGENTS

REGENSYS™

Regensys™ "A"

Regensys™ "A" is formulated as described by Maron & Ames, Mut. Res. 113: 173, 1983.
Size refers to the final volume of S9 mix.

60-200.15	15 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate
60-200.4	40 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate
60-200.5	50 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate

Regensys™ "B"

Regensys™ "B" consists of accurately weighed aliquots of crystalline NADP to add to corresponding Regensys™ "A" for completion of the Regensys™ system.

60-201.15L	46 mg (15 ml final volume)	1 each	Lyophilized NADP	Freezer
60-201.4L	123 mg (40 ml final volume)	1 each	Lyophilized NADP	Freezer
60-201.5L	153 mg (50 ml final volume)	1 each	Lyophilized NADP	Freezer

Regensys™ PLUS

Regensys™ "A" Plus

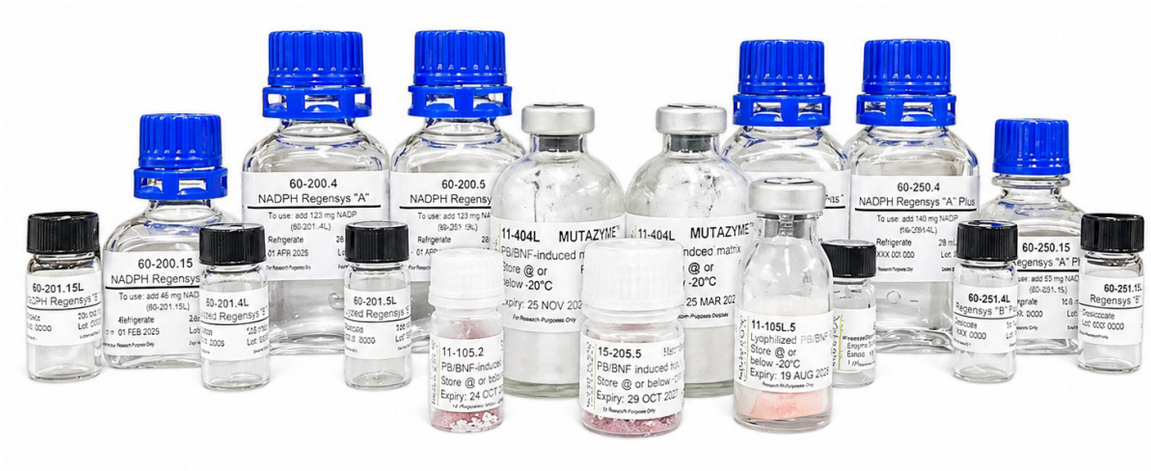
Regensys™ "A" PLUS is formulated for the preparation of
for 5-30% mixes. Ideal for use in the Enhanced Ames Test (EAT).
Size refers to the final volume of the S9 mix.

60-250.15	15 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate
60-250.4	40 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate
60-250.5	50 ml (final volume)	1 each	Glucose-6-Phosphate, Mg/KCl in 0.1 M Phosphate Buffer, pH 7.4	Refrigerate

Regensys™ "B" Plus

Regensys™ "B" PLUS consists of accurately weighed aliquots of crystalline NADP to add to corresponding Regensys™ "A" PLUS for completion of the Regensys™ PLUS system.

60-251.15L	53 mg (15 ml final volume)	1 each	Lyophilized NADP	Freezer
60-251.4L	140 mg (40 ml final volume)	1 each	Lyophilized NADP	Freezer
60-251.5L	175 mg (50 ml final volume)	1 each	Lyophilized NADP	Freezer



For more information, please refer to pages 57 -60.

Minimal Glucose Agars

Our Minimal Glucose Agars are specifically formulated for use in bacterial mutagenicity assays.
Along with the standard formulation as described by Maron & Ames, Mutat. Res. 113:173, 1983, MOLTOX® offers various other formulations, including different glucose concentrations.
Our MGAs are offered in case quantities or individual sleeves.

Minimal Glucose Agar

CASE QUANTITIES

21-400.5	30 ml	100 mm	2% Glucose	500 plates/case	Room Temp
21-40S10	25 ml	100 mm	0.4% Glucose	500 plates/case	Room Temp
21-40S21	25 ml	100 mm	2% Glucose	500 plates/case	Room Temp
21-40S29	25 ml	100 mm	0.4% Filter Sterilized Glucose	500 plates/case	Room Temp
21-40S65	28 ml	100 mm	0.4% Glucose	500 plates/case	Room Temp

Minimal Glucose Agar

SINGLE UNITS

21-400.2	30 ml	100 mm	2% Glucose	20 plates/sleeve	Room Temp
21-40S10.2	25 ml	100 mm	0.4% Glucose	20 plates/sleeve	Room Temp
21-40S19.10	5 ml/well	6 well	2% Glucose	10 plates/sleeve	Room Temp
21-40S284.10	5 ml/well	6 well	0.4% Glucose	10 plates/sleeve	Room Temp
21-40S294	1.3/ml well	24 well	0.4% Glucose	2 plates/sleeve	Refrigerate
21-40S300	1.25 ml/well	24 well	0.25 % Glucose	2 plates/sleeve	Refrigerate
21-40S307	1 ml/well	24 well	2% Glucose	2 plates/sleeve	Refrigerate
21-40S313.10	5 ml/well	6 well	L-Histidine & D-Biotin/ 2% Glucose	10 plates/sleeve	Room Temp
21-40S314.10	5 ml/well	6 well	L-Histidine & D-Biotin/ 0.4% Glucose	10 plates/sleeve	Room Temp
21-40S72	1 ml/well	24 well	0.4% Glucose	2 plates/sleeve	Refrigerate
26-686	600 ml	1 each	0.25 % Glucose	Glass Bottle	Room Temp

Top Agars

MOLTOX® prepared media and media components are specifically formulated for use in bacterial mutagenicity assays. Minimal Glucose agars, top agars, master plates, nutrient broth, and phenotype confirmation media are prepared with various formulations to meet specialized laboratory needs.

Standard Top Agar

26-501.1	100 ml	Glass Bottle	0.7% Agar	1 each	Room Temp
26-501.25	250 ml	Glass Bottle	0.7% Agar	1 each	Room Temp
26-501.3	300 ml	Glass Bottle	0.7% Agar	1 each	Room Temp
26-501.5	500 ml	Glass Bottle	0.7% Agar	1 each	Room Temp
26-501.6	600 ml	Glass Bottle	0.7% Agar	1 each	Room Temp
26-632.5	500 ml	Glass Bottle	0.6% Agar	1 each	Room Temp

Histidine/Biotin/Tryptophan Top Agar

26-721.1	100 ml	Glass Bottle	Histidine/Biotin/Tryptophan; 0.05 mM	1 each	Room Temp
26-721.25	250 ml	Glass Bottle	Histidine/Biotin/Tryptophan; 0.05 mM	1 each	Room Temp
26-721.5	500 ml	Glass Bottle	Histidine/Biotin/Tryptophan; 0.05 mM	1 each	Room Temp
26-721.75	750 ml	Glass Bottle	Histidine/Biotin/Tryptophan; 0.05 mM	1 each	Room Temp

L-Histidine/D-Biotin Top Agar

22-123	2 ml	Glass Tube	0.05 mM His/Bio; 0.7% Agar	10 tubes/box	Room Temp
26-503.1	100 ml	Glass Bottle	0.05 mM His/Bio; 0.7% Agar	1 each	Room Temp
26-503.3	300 ml	Glass Bottle	0.05 mM His/Bio; 0.7% Agar	1 each	Room Temp
26-503.5	500 ml	Glass Bottle	0.05 mM His/Bio; 0.7% Agar	1 each	Room Temp
26-545	500 ml	Glass Bottle	0.05 mM His/Bio; 0.6% Agar	1 each	Room Temp

L-Histidine/D-Biotin Top Agar

26-502.1	100 ml	Glass Bottle	0.05 mM Tryptophan; 0.7% Agar	1 each	Room Temp
26-502.3	300 ml	Glass Bottle	0.05 mM Tryptophan; 0.7% Agar	1 each	Room Temp
26-546	100 ml	Glass Bottle	0.05 mM Tryptophan; 0.6% Agar	1 each	Room Temp

Bacterial Tester Strains

The most commonly employed bacterial strains for use in mutagenicity testing are provided in convenient lyophilized disc format. Some strains are not amenable to freeze-drying and are offered either suspended in transport medium or frozen.

Strains are periodically characterized and verified for diagnostic phenotypes.

Confirmed strains are lyophilized in modified ATCC Reagent 18, suspended in transport medium (TA1535pSK1002), or frozen (TAMix). After preservation, samples are cultured and their phenotypes and responses to diagnostic mutagens are determined.

Strains are accompanied by CoA statements that include the results of phenotypic confirmation, diagnostic mutagen response and viability assessment.

Culture instructions are included with the CoA.

Note: The bacterial strains contained in STDiscs™ and ECDiscs™ are potential etiologic agents and are intended for use only by those skilled in the safe handling of potentially infectious agents.

Not offered below college level or to individuals. For research only.

*subculturing of these strains is not advisable

STDisc™ *Salmonella typhimurium* strains for use in the bacterial mutagenesis assay described by Maron & Ames, Mutat. Res. 113: 173, 1983.

Lyophilized STDisc™

71 - 097L	10 discs	Lyophilized	[*hisD6610*, *hisO1242*, *uvrB*, *rfa*, pKM101]	1 each	Refrigerate
71 - 098L	10 discs	Lyophilized	[*hisD3052*, *uvrB*, *rfa*, pKM101]	1 each	Refrigerate
71 - 100L	10 discs	Lyophilized	[*hisG46*, *uvrB*, *rfa*, pKM101]	1 each	Refrigerate
71 - 102L	10 discs	Lyophilized	[*hisG428*, *rfa*, pKM101, pAQ1]	1 each	Refrigerate
71 - 1535L	10 discs	Lyophilized	*[hisG46, uvrB, rfa]*	1 each	Refrigerate
71 - 1537L	10 discs	Lyophilized	*[hisC3076, uvrB, rfa]*	1 each	Refrigerate
71 - 1538L	10 discs	Lyophilized	*[hisD3052, uvrB ,rfa]*	1 each	Refrigerate
73 - 1535PSK	1 ml	Frozen	[*hisG46*, *uvrB*, *rfa*, pSK1002]	1 each	Ultra Low (-80°C)

Please visit our website or contact sales@moltox.com for additional 2 & 5 disc fill size information

ECDisc™ *Escherichia coli* strains (WP2 derivatives) for use in the bacterial mutagenesis assay described by Green & Muriel, Mutat. Res. 38: 3, 1976.

Lyophilized ECDisc™

72 - 002L	10 discs	Lyophilized	WP2 [*trpE*, pKM101]	1 each	Refrigerate
72 - 003L	10 discs	Lyophilized	WP2 [*trpE*, *uvrA*, pKM101]	1 each	Refrigerate
72 - 187L	10 discs	Lyophilized	WP2 [*trpE*]	1 each	Refrigerate
72 - 188L	10 discs	Lyophilized	WP2 [*trpE*, *uvrA*]	1 each	Refrigerate

Please visit our website or contact sales@moltox.com for additional 2 & 5 disc fill size information

TAMix consists of an equivocal mixture of *S. typhimurium* strains TA7000 through TA7006. The TA7000 series strains are responsive to each of the several possible base-pair substitution events.

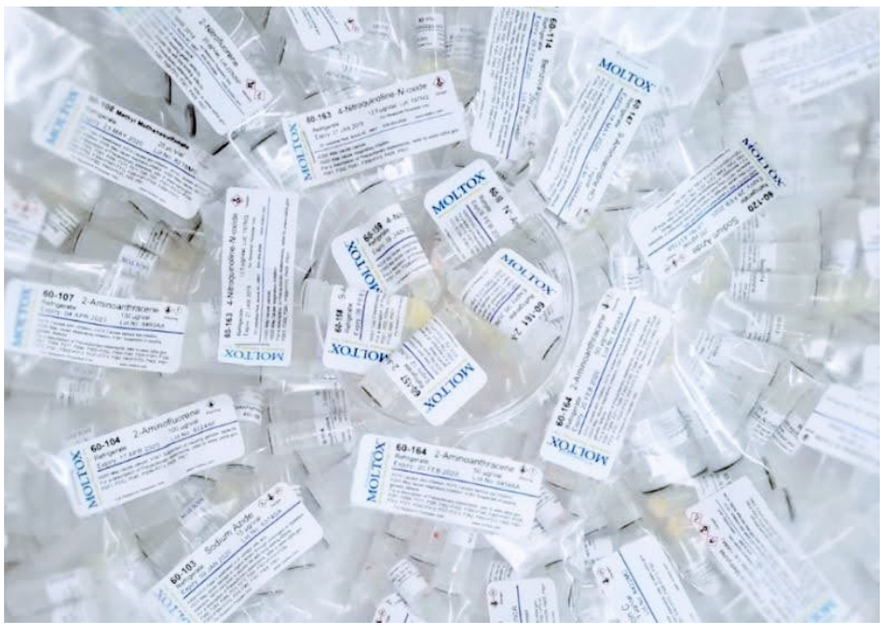
32 - 71001F	1 ml	Frozen	*Salmonella typhimurium* TA Mix Cells	1 each	Ultra Low (-80°C)

Positive Control Chemicals

MOLTOX® Positive Control Chemicals are obtained from major suppliers of research chemicals and are employed without further characterization or purification.

Packaged quantities are precise within 1%.

Please Note: All Positive Control Chemicals are known mutagens/carcinogens/toxins and are sold only to those experienced in the safe handling and disposal of hazardous materials. Consult your Institutional Safety Officer before ordering.



CONTROLCHEM™ chemicals are positive controls specifically packaged by MOLTOX® such that upon solubilization, the appropriate control dose is delivered in 0.1 ml volumes.

CONTROLCHEM™ chemicals are indicated in the following tables by *

For more information, please refer to pages 46 – 47.

DIRECT ACTING (-S9)

Direct acting mutagens do not require the use of S9 metabolic activation.

Mitomycin C

(CAS# 1404-00-8)

60-100*	5.0 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-100.6	50 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-100.7	150 µL/vial; 40 µg/ml	in dH2O	5 vials/pack	1 each	Refrigerate
60-100.10	10 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-100.11	100 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-100.20	20 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-100A100D	1mg/ml;100 µL/vial	in DMSO	5 vials/pack	1 each	Ultra Low (-80°C)
60-123	5 µg/ml ;1.0 ml/vial	in dH2O	5 vials/pack	1 each	Freezer

ICR 191

(CAS# 17070-45-0)

60-101*	10 µg/vial	Dry	5 vials/pack	1 each	Refrigerate

Daunomycin

(CAS# 20830-81-3)

60-102*	60 µg/vial	Dry	5 vials/pack	1 each	Refrigerate

Sodium Azide

(CAS# 26628-22-8)

60-103*	15 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-103.1	10 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-103.3	100 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-120	200 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-124	100 µg/vial	Dry	1 vial/pack	1 each	Refrigerate

9-aminoacridine-HCL

(CAS# 52417-22-8)

60-147*	1.0 mg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-147.5	500 µg /vial	Dry	5 vials/pack	1 each	Refrigerate
60-158	500 µg/vial	Dry	1 vial/pack	1 each	Refrigerate

Methyl methanesulfonate

(MMS; CAS# 66-27-3)

60-108*	25 µL /vial	Neat	5 vials/pack	1 each	Refrigerate
60-108.1	77 µL (100 mg)/vial	Neat	5 vials/pack	1 each	Refrigerate
60-108A25	1.0 mL/vial; 25 µL	in DMSO	5 vials/pack	1 each	Ultra Low (-80°C)

N^4–Aminocytidine

(CAS# 57294-74-3)

60-160	2.5 mg/vial	Dry	1 vial/pack	1 each	Refrigerate

Ethyl methane–sulfonate

(EMS; CAS# 62-50-0)

60-115*	20 µL/vial	Neat	5 vials/pack	1 each	Refrigerate

2–Nitrofluorene

(CAS#607-57-8)

60-111*	20 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-111.1	100 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-111.4	1 mg/vial	Dry	1 vial/pack	1 each	Refrigerate
60-161	50 µg/vial	Dry	1 vial/pack	1 each	Refrigerate

4–Nitroquinoline–N–oxide

(4-NQO; CAS# 56-57-5)

60-121.1	50 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-121.3	10 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-159	50 µg/vial	Dry	1 vial/pack	1 each	Refrigerate
60-163	12.5 µg/vial	Dry	1 vial/pack	1 each	Refrigerate

INDIRECT ACTING (+S9)

Indirect acting mutagens require the use of S9 metabolic activation.

2–Aminofluorene

(CAS# 153-78-6)

60-104*	100 µg/vial	Dry	5 vials/pack	1 each	Refrigerate

2–Aminoanthracene

(CAS# 613-13-8)

60-107*	100 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-107.2	200 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-107.21	20 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-107.5	500 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-157	100 µg/vial	Dry	1 vial/pack	1 each	Refrigerate
60-157.2	2.0 mg/vial	Dry	1 vial/pack	1 each	Refrigerate
60-164	50 µg/vial	Dry	1 vial/pack	1 each	Refrigerate

Danthron

(CAS#117-10-2)

60-122	500 µg/ml; 1.0 ml/vial	in DMSO	5 vials/pack	1 each	Freezer/Dark

Cyclophosphamide

(CAS# 6055-19-2)

60-113*	1.0 mg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-113.15	1.5 mg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-113.75	1.75 mg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-113A100	1.0 mg/mL;100 µL/vial	in DMSO	5 vials/pack	1 each	Ultra Low (-80°C)
60-119	1.0 mg/mL;100 µL/vial	in DMSO	5 vials/pack	1 each	Refrigerate

Benzo(a)pyrene

(CAS# 50-32-8)

60-114*	200 µg/vial	Dry	5 vials/pack	1 each	Refrigerate
60-114.2	2.5 mg/vial	Dry	1 vial/pack	1 each	Refrigerate
60-114.6	60 µg/vial	Dry	5 vials/pack	1 each	Refrigerate

7,12 - Dimethylbenz(a)anthracene

(DMBA; CAS# 57-97-6)

60-135	150 µg/vial	Dry	5 vials/pack	1 each	Refrigerate

Growth Media

MOLTOX® prepared media and media components are specifically formulated for use in bacterial mutagenicity assays. Minimal Glucose agars, top agars, master plates, nutrient broth and phenotype confirmation media are prepared with various formulations for specialized laboratory needs.

Nutrient Agar

Nutrient agars prepared with Oxoid Nutrient Broth No. 2, specified by Maron & Ames, Mutat. Res. 113: 173, 1983, are used for general growth and CFU determinations of Ames *S. typhimurium* and *E. coli* WP2 strains.

21-100	30 ml	100 mm plate	Oxoid #2	20 plates/sleeve	Room Temp
21-100.1	30 ml	100 mm plate	Oxoid #2	10 plates/sleeve	Room Temp
21-40S304.10	5 ml/well	6 well	Oxoid #2 w/VBE	10 plates/sleeve	Room Temp

Nutrient Broth

Nutrient Broth formulated with Oxoid Nutrient Broth No. 2 is primarily used in the Ames assay as specified by Maron &Ames, Mutat. Res. 113: 173, 1983 for culture of *S.typhimurium* and *E. coli* WP2.

26-505.025	25 ml	Plastic Bottle	Oxoid #2	1 each	Room Temp
26-505.05	50 ml	Plastic Bottle	Oxoid #2	1 each	Room Temp
26-505.1	100 ml	Glass Bottle	Oxoid #2	1 each	Room Temp
26-505.3	300 ml	Glass Bottle	Oxoid #2	1 each	Room Temp
26-505.5	500 ml	Glass Bottle	Oxoid #2	1 each	Room Temp
26-555.3	300 ml	Glass Bottle	Oxoid #2 w/ VBE	1 each	Room Temp
26-555.5	500 ml	Glass Bottle	Oxoid #2 w/ VBE	1 each	Room Temp

Phenotype Media

For phenotype confirmation of Ames strains and *E. coli* WP2 derivatives.

Each strain in the Ames assay should be phenotypically confirmed. These are a battery of tests to test for histidine/biotin auxotrophy (or tryptophan auxotrophy for *E. coli*), the presence/absence of the pKM101 plasmid (using ampicillin resistance), the *rfa* mutation, the *uvrB* or *uvrA* repair mutation (in the *Salmonella* and some of the *E. coli* strains, respectively), and the presence/abscene of pAQ1 plasmid (in TA102 only; using tetracycline resistance). [These tests are decribed on page 179 of the 1983 Ames paper and page 38 of the 2000 paper.]

ST QUAD PC™

21-200	5-6 ml/sector	Quad plate	MGA (I); MGA w/ His/Bio (II); MGA w/His/Bio + Amp (III); MGA w/His/Bio, Amp + Tet (IV)	5 plates/sleeve	Refrigerate

EC TRI PC™

21-199	8-9 ml/sector	Tri plate	MGA (I); MGA + Tryptophan (II); MGA + Tryptophan + Ampicillin (III)	5 plates/sleeve	Refrigerate

Phenotype Confirmation Well Plates

21-40S296	3.5 ml/well	6 well	For use with (26-300) Phenotype test packet (see pgs. 34 & 39)	2 plates/sleeve	Refrigerate

Histidine/Biotin Masters

21-203	30 ml	100 mm plate	MGA w/ His/Bio For non R-factor strains	10 plates/sleeve	Room Temp
21- 40S69	28 ml	100 mm plate	MGA w/ his/Bio For non R-factor strains	20 plates/sleeve	Room Temp

Ampicillin/Tetracyline Masters

21-202	30 ml	100 mm plate	Tetracycline masters : MGA w/ Histidine, Biotin Ampicillin & Tetracycline. For use with TA102 (pAQ1)	5 plates/sleeve	Refrigerate

Ampicillin Masters

21-201	30 ml	100 mm plate	MGA w/His/Bio + Amp For use w/ R-factor *S. typhimurium* strains	5 plates/sleeve	Refrigerate
21-40S39	25 ml	100 mm plate	MGA w/ Histidine, Biotin, Tryptophan & Ampicillin For use with R-factor *S. typhimurium* & *E.coli* strains	20 plates/sleeve	Refrigerate

Tryptophan UV Plates

21-40S70	28 ml	100 mm plate	With excess tryptophan For use with *E coli* WP2 strains	20 plates/sleeve	Room Temp

BACTERIAL MUTAGENESIS ASSAY KITS

MOLTOX®
Molecular Toxicology, Inc.

Salmonella
Mutagenicity Test Kit
Instruction Manual

31-100.2

MOLTOX®
Molecular Toxicology, Inc.

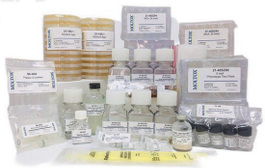
E. coli
Mutagenicity Test Kit
Instruction Manual

31-101

MOLTOX®
Molecular Toxicology, Inc.

µAmes
Mutagenicity Test Kit
Instruction Manual

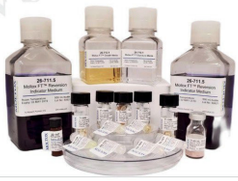
31-500



MOLTOX®
Molecular Toxicology, Inc.

Ames FT™
E. coli
Mutagenicity Test Kit
Instruction Manual

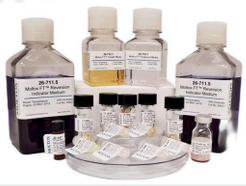
31-302



MOLTOX®
Molecular Toxicology, Inc.

Ames FT™
TA98/TA100
Mutagenicity Test Kit
Instruction Manual

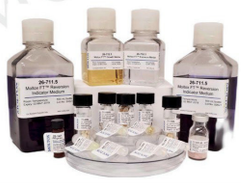
31-300



MOLTOX®
Molecular Toxicology, Inc.

Ames FT™
"471"
Mutagenicity Test Kit
Instruction Manual

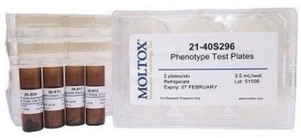
31-301



MOLTOX®
Molecular Toxicology, Inc.

Phenotype
Test Kit
Instruction Manual

31-600



MOLTOX®
Molecular Toxicology, Inc.

UMU Genotoxicity
Test Kit
Instruction Manual

31-400

Salmonella Mutagenicity Assay Kit

31-100.2

The materials contained in this *Salmonella* Mutagenicity Assay Kit include virtually all of the supplies necessary for the conduct of the "Ames Assay" as described by Maron & Ames [Maron, D. M. and B. N. Ames, Revised methods for the *Salmonella* mutagenicity test, Mutat. Res, 113: 173-215, 1983] and Mortelmans & Zeiger [Mortelmans, K. and E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res, 455: 29-60, 2000]. We strongly recommend that you carefully read one of these papers and OECD guideline 471 before you attempt to perform the assay. The MOLTOX® kit contains four tester strains; TA1535, TA1537, TA98, and TA100. Each strain was constructed with a different lesion in the histidine operon. This mutation renders them incapable of synthesizing histidine, i.e. they are histidine auxotrophs requiring exogenous histidine. In addition, TA1535, TA1537, TA98, and TA100 have altered cell walls (*rfa*) that increase the cell's permeability to certain high molecular weight materials. These strains also share a lesion in a DNA repair-coding gene (*uvrB*) which results in an increase in sensitivity to a variety of mutagens [since this lesion extends through the gene for biotin synthesis (*bio*), biotin is also required for growth]. Tester strains TA98 and TA100 carry a plasmid (pKM101) which acts to increase the activity of an error-prone DNA repair system and to confer resistance to the antibiotic ampicillin; TA1535 and TA1537 contain no plasmids. The MOLTOX® Salmonella Mutagenicity Test Kit includes sufficient components for testing 1 unknown sample, in triplicate analysis of 5 concentrations, positive and negative controls, with and without S9. MOLTOX® lyophilized S9 is freeze-dried phenobarbital/β-naphthoflavone-induced Sprague Dawley rat liver S9 as described by Matsushima, et al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

Description	PART #	Quantity
Lyoph. PB/BNF S9	11-05L.2	2
Nutrient Agar Dishes	21-100	1
ST QUAD PC™ plates	21-200	1
MGA Dishes	21-400.2	9
Nutrient Broth, 100 mL	26-505.1	1
His/Bio Top Agar, 100 mL	26-503.1	4
ICR 191, 10 µg/vial	60-101	1
Daunomycin, 60 µg/vial	60-102	1
Sodium Azide, 15 µg/vial	60-103	1
2-Aminoanthracene, 100 µg/vial	60-107	1
"Regensys A" , 40 mL	60-200.4	1
Lyoph. "Regensys B", 123 mg	60-201.4L	1
STDisc™ TA98, 5/vial	71-098.5L	1
STDisc™ TA100, 5/vial	71-100.5L	1
STDisc™ TA1535, 5/vial	71-1535.5L	1
STDisc™ TA1537, 5/vial	71-1537.5L	1

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

E. coli Mutagenicity Assay Kit

31-101

The materials contained in this *E. coli* Mutagenicity Assay Kit include virtually all of the supplies necessary for the conduct of the assay as described by Green & Muriel, Mutat. Res. 38: 3, 1976. We strongly recommend that you carefully read this paper before you attempt to perform the assay. The MOLTOX® *E. coli* kit contains two tester strains; WP2 (72-187L) and WP2 *uvrA* (72-188L). Each strain was constructed with a lesion (tryptophan mutation) in the tryptophan operon. WP2 *uvrA* also contains a lesion in a DNA repair-coding gene (*uvrA*) that increases sensitivity to certain mutagenic activities. The MOLTOX® *E. coli* Mutagenicity Test kit includes sufficient components for testing 1 unknown sample, in triplicate analysis of 5 concentrations, positive and negative controls, with and without S9. MOLTOX® lyophilized S9 is freeze-dried phenobarbital/β-naphthoflavone induced Sprague Dawley rat liver S9 as described by Matsushima, et al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

Description	PART #	Quantity
Lyoph. PB/BNF S9	11-05L.2	1
Nutrient Agar Dishes	21-100	1
EC TRI PC™ Dishes	21-199	1
MGA Dishes	21-400.2	4
Nutrient Broth, 100 mL	26-505.1	1
Tryptophan Agar, 100 mL	26-502.1	2
2– Aminoanthracene, 100 µg/vial	60-107	1
Methyl Methanesulfonate, 25 µL/vial	60-108	1
"Regensys A", 15 mL	60-200.15	1
Lyoph. "Regensys B", 46 mg	60-201.15L	1
ECDisc™ WP2, 5/vial	72-187.5L	1
ECDisc™ WP2 (*uvrA*) 5/vial	72-188.5L	1

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® µAmes Kit

31-500

This kit allows the Ames bacterial mutation test described in OECD guideline 471 to be performed in a miniature, 24-well plate format. This version is ideal for non-GLP evaluation of genotoxicity, as a pre-screen at an early stage of compound development or when test material supply is limited or for training purpose. The microAmes (µAmes) version of the test accurately predicts the outcome of the subsequent GLP study with similar sensitivity, uses a very low amount of test article (often important during early development), and can be performed by an individual on a single day without special equipment. Since both the standard and µAmes method use nearly the same procedures, results from the micro method can be extrapolated to those expected for the standard Ames test. The MOLTOX® µAmes Kit includes sufficient components for testing 1 unknown sample, in triplicate analysis of 8 concentrations, positive and negative controls, with and without S9. MOLTOX® 10% PB/BNF Mutazyme™ is a freeze-dried product that contains phenobarbital/β-naphthoflavone induced Sprague Dawley rat liver S9 and S9 mix cofactors as described by Mortelmans and Zeiger

Description	PART #	Quantity
10 % PB/BNF Mutazyme™ , 20 mL	11-404L	1
Nutrient Agar Dishes 10/sleeve	21-100.1	2
6 well PC plates	21-405296	3
MGA Dishes 24-well plates	21-405294	11
Phenotype Test Packet (26-810, 26-811, 26-812, 26-813)	26-300	1
Nutrient Broth, Oxoid No. 2, 25 mL	26-505.025	6
Phosphate Buffer 0.1M pH 7.4, 100 mL	26-543.039	1
Deionized Water, 25 mL	26-682	1
Top Agar, 0.05 mM His/Bio/Tryp, 100 mL	26-721.1	1
Ames Control Chem Packet (60-103.1, 60-107.21, 60-107.2, 60-111, 60-114.6, 60-121.3, 60-147.5)	60-300	1
Bacterial Strain Packet (71-098.2L, 71-100.2L, 72-188.2L, 71-1535.2L, 71-1537.2L)	71-300	1

(Mortelmans, K. and E. Zeiger, The Ames *Salmonella/* microsome mutagenicity assay. Mutat. Res., 455: 29-60, 2000); induction as described by Matsushima, et. al., (In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85).

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

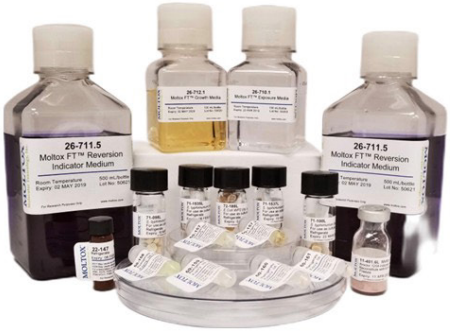
SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® FT™ "471" Mutagenicity Assay Kit

31-301

The MOLTOX® "471" FT™ test measures the ability of test material treatments to induce reversion of histidine requiring *Salmonella typhimurium* strains or *Escherichia coli* tryptophan auxotrophs to their respective prototrophic conditions. The bacterial strains used in these assays are identical to those used in conventional plate incorporation assays as described by Mortlemans & Zeiger, Mutat. Res. 455: 29, 2000 and Mortlemans & Riccio, Mutat. Res. 455: 61, 2000. The experimental design used in the MOLTOX® FT™ tests is based on Gatehouses's adaption of the design reported by Luria & Delbruck, Mutat. Res. 53: 289, 1978 and Genetics, 28:491, 1943. The MOLTOX® FT™ "471" Mutagenicity Assay kit contains the products required to perform a microtiter fluctuation test using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 & *E. coli* WP2 *uvrA*. This kit includes sufficient components for 1 test material in triplicate analysis of 6 concentrations,

Description	PART #	Quantity
30% PB/BNF Mutazyme™, 3.25 mL	11-406.3L	2
Ampicillin, 55 mg/vial	22-147	1
FT™ Exposure Media, 100 mL	26-710.1	1
FT™ Reversion Indicator Media, 500 mL	26-711.5	2
FT™ Growth Media, 100 mL	26-712.1	1
2-Aminoanthracene, 100 µg/vial	60-157	1
2-Aminoanthracene, 2 mg/vial	60-157.2	1
9-Aminoanthracene hydrochloride, 500 µg/vial	60-158	1
4-Nitroquinoline-*N*-oxide, 50 µg/vial	60-159	1
N^4-aminocytidine, 2.5 mg/vial	60-160	1
2-Nitrofluorene, 50 µg/vial	60-161	1
STDisc™ TA98, 2/vial	71-098.2L	1
STDisc™ TA100, 2/vial	71-100.2L	1
STDisc™ TA1535, 2/vial	71-1535.2L	1
STDisc™ TA1537, 2/vial	71-1537.2L	1
ECDisc™ WP2 *uvrA*, 2/vial	72-188.2L	1



positive and negative controls, with and without S9. Assay is consistent with OEDC 471. MOLTOX® 30% PB/BNF Mutazyme™ is a freeze-dried product that contains phenobarbital/β-naphthoflavone induced Sprague Dawley rat liver S9 and S9 mix cofactors as described by Mortelmans and Zeiger (Mortelmans, K. and E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res., 455: 29-60, 2000); induction as described by Matsushima, et. al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® FT™ *E. coli* Mutagenicity Assay Kit

31-302

The MOLTOX® FT™ *E. coli* test measures the ability of test material treatments to induce reversion of *Escherichia coli* tryptophan auxotrophs to their prototrophic condition. The bacterial strains used in these assays are identical to those used in conventional plate incorporation assays as described by Mortelmans & Riccio, Mutat. Res. 455: 61, 2000. The experimental design used in the MOLTOX® FT™ tests is based on Gatehouses's adaption of the design reported by Luria & Delbruck, Mutat. Res. 53: 289, 1978 and Genetics, 28: 491, 1943. The MOLTOX® FT™ *E. coli* Mutagenicity Assay Kit consists of materials to perform a microtiter fluctuation test using *Escherichia coli* WP2 *pKM101* and WP2 *uvrA*. This assay includes sufficient components for 1 test material in triplicate analysis of 6 concentrations, positive and negative controls, with and without S9. MOLTOX® 30% PB/BNF Mutazyme™ is a freeze-dried product that contains phenobarbital/β-naphthoflavone induced Sprague Dawley rat liver S9 and S9 mix cofactors as described by Mortelmans and Zeiger (Mortelmans, K. and E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res., 455: 29-60, 2000); induction as described by Matsushima, et. al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

Description	PART #	Quantity
30% PB/BNF Mutazyme™, 3.25 mL	11-406.3L	1
Ampicillin, 55 mg/vial	22-147	1
FT™ Exposure Media, 50 mL	26-710.05	1
FT™ Reversion Indicator Media, 150 mL	26-711.15	2
FT™ Growth Media, 50 mL	26-712.05	1
2-Aminoanthracene, 2 mg/vial	60-157.2	1
4-Nitroquinoline-*N*-oxide, 50 µg/vial	60-159	1
ECDisc™ WP2 *pKM101*, 2/vial	72-002.2L	1
ECDisc™ WP2 *uvrA*, 2/vial	72-188.2L	1

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® FT™ TA98/TA100 Mutagenicity Assay Kit

31-300

The MOLTOX® FT™ TA98/TA100 test measures the ability of test material treatments to induce reversion of histidine requiring *Salmonella typhimurium* TA98 and TA100 strains to their respective prototrophic conditions. The bacterial strains used in this assay are identical to those used in conventional plate incorporation assays as described by Mortelmans & Zeiger, Mutat. Res. 455: 29, 2000. The experimental design used in the MOLTOX® FT™ tests is based on Gatehouses's adaption of the design reported by Luria & Delbruck, Mutat. Res. 53: 289, 1978 and Genetics, 28: 491, 1943. The MOLTOX® FT™ TA98 & TA100 Mutagenicity Assay Kit includes sufficient components for 1 test material in triplicate analysis of 6 concentrations, positive and negative controls, with and without S9. MOLTOX® 30% PB/BNF Mutazyme™ is a freeze-dried product that contains phenobarbital/β-naphthoflavone induced Sprague Dawley rat liver S9 and S9 mix cofactors as described by Mortelmans and Zeiger (Mortelmans, K. and E. Zeiger, The Ames *Salmonella*/microsome mutagenicity assay. Mutat. Res., 455: 29-60, 2000); induction as described by Matsushima, et. al., [In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85].

Description	PART #	Quantity
30% PB/BNF Mutazyme™, 3.25 mL	11-406.3L	1
Ampicillin, 55 mg/vial	22-147	1
FT™ Exposure Media, 50 mL	26-710.05	1
FT™ Reversion Indicator Media, 150 mL	26-711.15	2
FT™ Growth Media, 50 mL	26-712.05	1
2-Aminoanthracene, 100 µg/vial	60-157	1
4-Nitroquinoline-*N*-oxide, 50 µg/vial	60-159	1
2-Nitrofluorene, 50 µg/vial	60-161	1
STDisc™ TA98, 2/vial	71-098.2L	1
STDisc™ TA100, 2/vial	71-100.2L	1

Reconstitute to the label volume using ice cold, sterile, purified water – maintain on ice.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® UMU Genotoxicity Test Kit

31-400

The MOLTOX® UMU Genotoxicity Test Kit protocol was adapted from ISO 13829 "Water Quality Determination of the genotoxicity of water and waste water using the UMU test". The MOLTOX® UMU Kit provides components to perform both aqueous and chemical tests based on the user's needs. This includes sufficient materials to test for 6 unknowns in triplicate analysis of 4 concentrations, with and without S9. *Salmonella typhimurium* TA1535 (*hisG46, rfa, uvrB*) has been modified to contain the plasmid pSK1002. This plasmid contains the gene *umuC* fused to a *lacZ* reporter gene. If genetic lesions are formed when exposed to potentially genotoxic compounds, the *umuC* gene is induced as part of the bacterial SOS response. Due to the *lacZ-umuC* fusion and the accompanying *lacZ*-encoded β-galactosidase activity, genotoxic induction can be detected by the colorimetric change of ONPG substrate (colorless) to 2-nitrophenol (yellow).

Description	PART #	Quantity
30 % PB/BNF Mutazyme™, 3.25 mL	11-406.3L	1
Ampicillin, 55 mg	22-147	1
ONPG, 45 mg/mL reconstituted	22-148	2
2-Mercaptoethanol, 0.1 mL	22-149	2
TGA Culture Media, 100 mL	26-714	2
10X TGA Culture Media, 10 mL	26-715	1
B-Buffer, 35 mL	26-716	1
Stop Reagent, 30 mL	26-718	1
4-NQO; 12.5 µg/vial	60-163	1
2-AA; 50 µg/vial	60-164	1
S. typhimurium TA1535/pSK1002 1 mL	73-1535pSK	2

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

Phenotype Test Kit

31-600

The MOLTOX® Phenotype Test Kit has been specially designed to confirm all the phenotype characteristics listed by OEDC in any of the *E. coli* and *Salmonella* strains used in the Ames test including: Amino-acid requirement, presence/absence of the pKM101 R-factor plasmid, presence/absence of the pAQ1 plasmid, *rfa* deep mutation, *uvrA* and *uvrB* repair deficiency. This test uses a single plate per strain, takes only a few minutes and can be performed alongside a standard Ames test using any type of agar. This kit provides enough material for 6 tests. Results are available the next day.

Description	PART #	Quantity
6 well Phenotype Plates	21-40S296	3
Phenotype Test Packet 26-810 Ampicillin discs 26-811 Tetracycline discs 26-812 Mitomycin C discs 26-813 Crystal Violet discs	26-300	1

The Phenotype Test Kit is manufactured using the highest quality components, material preparation, strain characterization and procedures closely follow formative guidelines. Each batch of materials is thoroughly tested before release and is accompanied by C of As.

SEE PREVIOUS PAGES FOR PURCHASE OF INDIVIDUAL ITEMS & FILL SIZES

MOLTOX® FT™ Components for individual purchase

MOLTOX® offers all the components of our kits separately so you can custom design your assays by purchasing the exact amount of materials needed to perform your analysis.

Exposure Media

26-710.05	50 ml	Plastic Bottle	Exposure Medium for use in MOLTOX® FT kit	1 each	Room Temp
26-710.1	100 ml	Plastic Bottle	Exposure Medium for use in MOLTOX® FT kit	1 each	Room Temp
26-710.25	250 ml	Plastic Bottle	Exposure Medium for use in MOLTOX® FT kit	1 each	Room Temp

Growth Media

26-712.05	50 ml	Plastic Bottle	Growth Media for use in MOLTOX® FT kit	1 each	Room Temp
26-712.1	100 ml	Plastic Bottle	Growth Media for use in MOLTOX® FT kit	1 each	Room Temp
26-712.25	250 ml	Plastic Bottle	Growth Media for use in MOLTOX® FT kit	1 each	Room Temp
26-715	10 ml	Glass Vial	10X TGA Growth Media for use in MOLTOX® FT kit	1 each	Room Temp

Antibiotics

22-147	55 mg	Amber Vial	Ampicillin. For use with MOLTOX® FT kits	1 each	Refrigerate

For Bacterial Discs and Positive Control Chemicals, please refer to pages 18, 19, and 20-25.

How do I plan my material needs for an Ames assay?

As many genetic toxicologists will tell you, the bulk of the work in an Ames assay is in the planning and set up of the assay itself. This MOLTOX® Technical Bulletin will assist you in planning considerations and determination of Ames assay material needs.

Planning Questions:

- How many sample doses are you going to test?
  - The OECD 471 guideline, "Bacterial Reverse Mutation Test", requires 5 analyzable doses. Many researchers choose to test 8 doses to capture this data. If not following the OECD 471 guideline, a minimum of 5 doses should be considered.
  - The upper dose should not exceed 5 mg/plate. Select doses separated by factors of 2, 3 (or approximate half logs, the OECD 471 recommendation), or 5.
- How many strains will you use?
  - If performing a screening assay the primary strains used are TA98 and TA100. These strains detect frameshift and base-pair substitution mutations, respectively.
  - If following the OECD 471 guideline, 5 strains are required. The recommended combination of strains is:

    S. typhimurium TA1535, and

    S. typhimurium TA1537 or TA97 or TA97a, and

    S. typhimurium TA98, and

    S. typhimurium TA100, and

    E. coli WP2 *uvrA*, or *E. coli* WP2 *uvrA* (pKM101), or S. *typhimurium* TA102.
- Will you perform the assay in duplicate or triplicate per dose?
- Will you perform the assay both with and without S9?
  - If using S9, what concentration of S9 mix will you use? 10% is the most common, followed by 5%.
- Will you need to perform a cell titer test to verify your culture density or do you have previous growth curve data connecting culture density to an OD range?
- What positive controls will be needed?
  - Positive controls are, for the most part, strain specific.
  - Tests performed with S9 require a +S9 positive control, tests performed without S9 require a -S9 positive control.
- How many plates can your lab realistically process in a day?
  - This is dependent upon experience, # of strains used, + and/or -S9 conditions, # of doses/test sample, duplicate or triplicate plating, etc.
  - Some of the reagents must be used the day of testing. If more than one day of testing is needed, additional reagents may be needed.

Determining Material Needs per Test Sample

Number of Minimal Glucose Agar (MGA) plates -

# of test doses + **2** (positive and vehicle/negative controls) = **A**

# of tester strains = **B**

Duplicate or triplicate plating = **C** (2 or 3)

+S9, -S9, or +/- S9 = **D** (l for+ S9 or -S9, 2 if +/-S9)

A x B x C x D = # of Minimal Glucose Agar plates needed

Suggested MOLTOX® Products	
21-400.2	20 MGA Plate/Sleeve
21-400.5	500 MGA Plates/Case

Volume of Top Agar -

◊ *S. typhimurium* strains require 0.05mM Histidine/Biotin top agar; *E. coli* strains require 0.05mM Tryptophan top agar. 2 mls/MGA plate is required.

◊ For *S. typhimurium* portion of test:

# of test doses + 2 (positive and negative controls)= **E**

of *S. typhimurium* strains= **F**

Duplicate or triplicate plating= **G** (2 or 3)

+S9, -S9, or +/- S9 = **H** (1 for + S9 or -S9, 2 if +/- S9)

(E x F x G x H) x 2 = Volume (mls) of 0.05mM Histidine/Biotin top agar needed

◊ For *E. coli* portion of test:

# of test doses + 2 (positive and negative controls) = I

of *E. coli strains* = **J**

Duplicate or triplicate plating= **K** (2 or 3)

+S9, -S9, or +/- S9 = **L** (1 for + S9 or -S9, 2 if +/- S9)

(I x J x K x L) x 2 = Volume (mls) of 0.05mM tryptophan top agar needed

Suggested MOLTOX® Products	
26-503.1	0.05mM Histidine/Biotin top agar, 100 mls
26-503.3	0.05mM Histidine/Biotin top agar, 300 mls
26-503.5	0.05mM Histidine/Biotin top agar, 500 mls
26-502.1	0.05mM Tryptophan top agar, 100 mls
26-502.3	0.05mM Tryptophan top agar, 300 mls
26-721.1	0.05mM Histidine/Biotin/Tryptophan top agar, 100 mls
26-721.25	0.05mM Histidine/Biotin/Tryptophan top agar, 250 mls
26-721.5	0.05mM Histidine/Biotin/Tryptophan top agar, 500 mls
26-721.75	0.05mM Histidine/Biotin/Tryptophan top agar, 750 mls

Volume of S9 Mix-

- ◊ +S9 plates require 0.5 mls of S9 mix/MGA plate.
- ◊ For an assay performed both **with and without** S9, ½ the number of MGA plates determined above require S9 mix. Therefore;

  (# of MGA plates needed)/2 x 0.5 mls = Volume S9 mix needed

- ◊ For an assay performed **with S9 only,** all the MGA plates determined above require S9 mix. Therefore;

  # of MGA plates needed x 0.5 mls = Volume S9 mix needed

Suggested MOLTOX® Products	
60-200.15	Regensys™ A, 15 mls *
60-200.4	Regensys™ A, 40 mls *
60-200.5	Regensys™ A, 50 mls *
60-201.15L	Regensys™ B, 46 mg
60-201.4L	Regensys™ B, 123 mg
60-201.5L	Regensys™ B, 153 mg
11-404L	Mutazyme™, 10%, 20 mls
11-405L	Mutazyme™, 5%, 20 mls
* Final volume of S9 mix upon addition of S9 or S9 and sterile dH_2O	

Volume of S9 -

The volume of S9 required is determined by the total volume of S9 mix needed for the assay and the concentration of S9 mix desired. Therefore;

Volume of S9 mix (mls) x % S9 mix desired (in decimals) = mls S9 needed

Ex. 80 mls S9 mix x 0.10 (10%) = 8 mls S9

Suggested MOLTOX® Products	
11-105.1	Phenobarbital/β-Naphthoflavone induced S9, 1 ml/vial
11-105.2	Phenobarbital/β-Naphthoflavone induced S9, 2 ml/vial
11-105.5	Phenobarbital/β-Naphthoflavone induced S9, 5 ml/vial
11-05L.1	Phenobarbital/β-Naphthoflavone induced S9, 1 ml/vial; lyophilized
11-05L.2	Phenobarbital/β-Naphthoflavone induced S9, 2 ml/vial; lyophilized
11-05L.5	Phenobarbital/β-Naphthoflavone induced S9, 5 ml/vial; lyophilized

Other Media Needs

◊ Oxoid Nutrient Broth No. 2 (ONB#2)

ONB#2 is essential for strain growth. Do not substitute other products.

Fresh cultures of each tester strain are needed for each day of testing.

The volume used/strain is up to the researcher. For overnight growth, 25 - 30 mis is recommended. Assuming overnight growth;

30 mls x # of tester strains x #of test days = Volume ONB#2 needed

◊ Phenotype plates

If required by your institution, phenotype testing of the strains may be necessary. Depending on plate format, 1- 4 strains may be tested/plate.

◊ Oxoid Nutrient No. 2 Agar plates

ONB#2 agar plates are necessary if performing overnight titer tests to confirm a cell density of 1- 2 x10E^{9}cfu/ml in the cultures used.

The number of plates needed is dependent on the # of dilutions plated and if the plating is performed in duplicate or triplicate.

Generally, 6 plates are sufficient/strain (2 dilutions in triplicate or 3 dilutions in duplicate).

Suggested MOLTOX® Products	
26-505.1	Oxoid Nutrient Broth No. 2, 100 mls
26-505.3	Oxoid Nutrient Broth No. 2,300 mls
26-505.5	Oxoid Nutrient Broth No. 2, 500 mls
21-199	EC Tri PC™ Plates, 5/sleeve
21-200	ST Quad PC™ Plates, 5/sleeve
31-600	Phenotype Test Kit
21-100	Oxoid Nutrient Broth No. 2 Agar Plates, 20/sleeve

Positive Controls

The below are suggested positive controls for each strain and the recommended dose/plate.

Positive Controls for Use Without S9

Strain		Positive Control	Dose/plate (µg)
TA1535		Sodium Azide	5
	OR	N^4-Aminocytidine	250
TA1537		9-Aminoacridine HCl	50
	OR	ICR 191	1
TA1538		Daunomycin	6
	OR	2-Nitrofluorene	2
TA97a		9-Aminoacridine HCl	50
	OR	ICR 191	1
TA98		Daunomycin	6
	OR	2-Nitrofluorene	2
TA100		Sodium Azide	5
	OR	N^4-Aminocytidine	250
TA102		Mitomycin C	0.5
E.coli WP2 *E.coli* WP2 *uvrA* *E.coli* WP2 pKM101 *E.coli* WP2 *uvrA* pKM101		Methyl methanesulfonate (MMS)[A]	2.5
[A] MMS is a *neat* (i.e., liquid) chemical. Dose is 2.5 µl/plate			

Positive Controls for Use With S9

Strain		Positive Control	Dose/plate (µg)
TA1535		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[b]	10
	OR	Cyclophosphamide	100
	OR	Benzopyrene	20
	OR	7,12 - Dimethylbenz(a)anthracene[c]	10
TA1537		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[b]	10
TA1538		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[b]	10
	OR	Benzopyrene	20
TA97a		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[d]	5
TA98		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[d]	5
	OR	Benzopyrene	20
TAI00		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[d]	5
	OR	Cyclophosphamide	100
	OR	Benzopyrene	20
	OR	7,12 - Dimethylbenz(a)anthracene[c]	10
TA102		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[c]	10
	OR	Danthron	50
E.coli WP2 *E. coli* WP2 *uvrA* *E.coli* WP2 pKM101 *E. coli* WP2 *uvrA* pKM101	OR	2-Aminofluorene[a] 2-Aminoanthracene[a]	20 20

a Suggested dose is 10 - 20 µg/plate

b Suggested dose is 2 - 10 µg/plate

c Suggested dose is 5 - 10 µg/plate

d Suggested dose is 1 - 5 µg/plate

Standard OECD Ames Test vs. Enhanced Ames Test EAT Protocol

Condition	Standard OECD Ames Test	Enhanced Ames Test
Tester Strains	*S. typhimurium* TA98 TA100 TA1535 TA1537 or TA97 or TA97a *E. coli* WP2 *uvrA or* WP2 *uvrA* (pKM101) **or** *S. typhimurium* TA102	*S. typhimurium* TA98 TA100 TA1535 TA1537 *E. coli* WP2 *uvrA* (pKM101)
Protocol	Preincubation (20 minutes incubation) **or** Plate incorporation	Preincubation (30 minutes incubation)
Metabolic Activation	5-30% S9 prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254 or a combination of phenobarbital and β-naphthoflavone, and in the absence of S9.	30% rat liver S9, 30% hamster liver S9, as well as in the absence of S9. S9 should be prepared from rodents treated with inducers of cytochrome P450 enzymes (e.g., a combination of phenobarbital and β-naphthoflavone).
Solvent/Negative Control	Water/Organic Solvent	Water/Organic Solvent (the lowest possible volume should be included in the pre-incubation mixture with justification to indicate that the volume of solvent does not interfere with metabolic activation of the *N*-nitrosamine).
Positive Control	Concurrent Strain-Specific Positive Controls	In addition to Concurrent Strain-Specific Positive Controls, two *N*-nitrosamines that are known to be mutagenic in the presence of S9 should be included, the choice of which should be justified based on the anticipated metabolism of the *N*-nitrosamine and cytochrome P450 enzyme most likely involved.

MOLTOX®
Molecular Toxicology, Inc.

Growing Broth Cultures using MOLTOX®

ST- and EC- Discs™

This guidance is for growing MOLTOX® lyophilized *S. typhimurium* and *E. coli* bacterial discs for use in the bacterial reverse mutation assay (i.e., Ames Assay).

General Information on growing these strains-

Nutrients

Oxoid Nutrient Broth No. 2 is **required** to grow these bacterial strains. Other brands of nutrient broth do not perform as well and may actually be detrimental to the culture resulting in inaccurate results.

Oxygen

Grow these organisms aerobically. Use a vessel that is 3 - 5x the volume of broth. Ensure adequate aeration by leaving the vessel cap or plug loose and orienting your vessel to achieve maximum broth surface area (i.e., slant tubes if using).

Incubation Conditions

Grow these cultures in a dry incubator at 35 ± 2°C.
Shaking the culture increases aeration and decreases overall incubation time.
Grow these cultures to a density of 1-2 x $10E^{9}$ cfu/ml. **Generally,** this is equal to an OD_{650nm}= 1.0 - 1.4. Ideally this would be confirmed with a growth curve study in the user's laboratory. Density can be confirmed through serial dilution and plating. These cultures are used in the active growing phase. Use them **as they enter** the above density.

Directions for Use:

On the evening prior to use in testing, warm the product vial to room temperature[1]; using sterile forceps or loop, aseptically transfer a disc to 25 - 30 mls Oxoid Nutrient Broth #2. Hold the culture **stationary** at 37°C overnight[2]. Early the next morning, incubate with shaking at 37°C until a density of 1 - 2 x 10^{9} bacteria/ml is achieved, at which point the culture will be virtually opaque. Under optimal conditions this bacteria may double in number approximately every 30 minutes or less. Do not overgrow the cultures[3].

[1] This prevents condensation from forming in the vial which could impair the performance of the discs.

[2] The number of viable cells in the discs is high enough that, if the culture is shaken overnight, the culture will overgrow and be out of the active growing phase.

[3] If the culture is overgrown, **do not** dilute such culture to the recommended range. Instead, remove a portion of the culture, add to fresh Oxoid Nutrient Broth No. 2 and incubate with shaking, monitoring until the culture enters the recommended density.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Lyophilized Culture Discs
(71- and 72- part numbers)

Intended Use:

For use in the *in vitro* Bacterial Reverse Mutation Test; i.e., Ames Assay. See below table to determine the reversion event that each strain detects and for plasmid information.

Warnings and Precautions:

For Laboratory Use.

The bacterial strains contained in the lyophilized discs are potential etiologic agents and are intended for use only by those skilled in the safe handling of potentially infectious agents. The strains are considered BioSafety Level 2 organisms and should be handled accordingly [see CDC/NIH Biosafety in Microbiological and Biomedical Laboratories, HHS Publication {CDC) 93-8395. Available from US Government Printing Office, Superintendent of Documents, Washington DC 20402 {Stock No. 017-040-00523-7)].

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

After use, dispose of all materials according to your institutional biohazard program.

Storage:

Upon receipt, store discs tightly closed under refrigeration at 2 - 8°C.

Procedure:

Warm the product vial to room temperature and remove the cap and stopper so as to avoid contaminating the assembly. Using a flamed or disposable bacteriological loop, needle, or tweezers, aseptically remove a disc and use to inoculate a quantity of Oxoid #2 nutrient broth. Use a vessel that is 3 - 5 times the volume of the culture to ensure adequate aeration. Leave the cap loose for adequate oxygen exchange. Orient the vessel such that maximum surface area is achieved with the broth. Hold the culture stationary at 37°C overnight. Early the next morning incubate with shaking at 37°C until a density of 1 - 2 x 10^9 bacteria per ml is achieved, at which point the culture will be virtually opaque - the density can be estimated by direct counting, measurement of optical density or plating dilutions on nutrient agar. Under optimal conditions this bacteria may double in number approximately every 30 minutes or less. Do not overgrow the culture(s) as this may lead to erroneous results.

MOLTOX®
Molecular Toxicology, Inc.

S. typhimurium and *E. coli* WP2 strains

Part Number	Strain Designation	Reversion Event	Plasmid	Antibiotic resistance
71-1535L	*S. typhimurium* TA1535	Base-pair substitution	N/A	N/A
71-1537L	*S. typhimurium* TA1537	Frameshift	N/A	N/A
71-1538L	*S. typhimurium* TA1538	Frameshift	N/A	N/A
71-100L	*S. typhimurium* TA100	Base-pair substitution	pKM101	Ampicillin
71-097L	*S. typhimurium* TA97a	Frameshift	pKM101	Ampicillin
71-098L	*S. typhimurium* TA98	Frameshift	pKM101	Ampicillin
71-102L	*S. typhimurium* TA102	Base-pair substitution Crosslinks	pKM101, pAQ1	Ampicillin, Tetracycline
72-187 L	*E. coli* WP2 *trp*	Base-pair substitution Crosslinks	N/A	N/A
72-188L	*E. coli* WP2 *trp uvrA*	Base-pair substitution	N/A	N/A
72-002 L	*E. coli* WP2 *trp* pKM101	Base-pair substitution Crosslinks	pKM101	Ampicillin
72-003L	*E.* coli WP2 *trp uvrA* pKM101	Base-pair substitution	pKM101	Ampicillin

Expected Results:

Refer to the MOLTOX® lot specific C of A statement, internal laboratory historical data and/or your institutional SOP for spontaneous and positive control treatment reversion rates.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

ST QUAD PC™ Plates (21-200)

Intended Use:

For the phenotype confirmation testing of *S. typhimurium* "Ames" strains.

This plate tests for:

- histidine auxotrophy *(his⁻),*
- sensitivity to crystal violet (*rfa*),
- presence of plasmid pKM101 (Amp^R) and,
- presence of plasmid pAQ1 (Tet^R).

Storage:

Upon receipt, store plates at 2 - 8°C.

Procedure:

Using a sterile 10µL inoculating loop or a swab, streak *S. typhimurium* broth culture onto each sector of the plate. If streaking with a swab, remove excess broth against the side of the growth vessel prior to streaking.

Using sterile forceps, apply the supplied crystal violet disc to the center of the streak in Sector 2 (II, B).

Incubate overnight at 37°C.

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

After use, dispose of all materials according to your institutional biohazard procedures.

Expected Results:

Sector		Strain Response		
Designation	**Description**	**TA1535 TA1537 TA1538**	**TA97a TA98 TA100**	**TA102**
1 (I, A)	MGA + Biotin	-	-	-
2 (II, B)	MGA + Histidine/Biotin	+	+	+
3 (III, C)	MGA + Histidine/Biotin/Ampicillin	-	+	+
4 (IV, D)	MGA +Histidine/Biotin/Ampicillin/Tetracycline	-	-	+

All strains should exhibit a halo around the crystal violet disc (i.e., no growth in the presence of crystal violet).

MOLTOX®
Molecular Toxicology, Inc.

Product Information

EC Tri PC™ Plates (21-199)

Intended Use:

For the phenotypic confirmation of *E. coli* WP2 strains used in the Bacterial Reverse Mutation Assay (i.e., Ames Assay).

This plate tests for:

- tryptophan auxotrophy (*trp*$^-$)
- presence of plasmid pKM101 (Amp^R).

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

After use, dispose of all materials according to your institutional biohazard program.

Storage:

Upon receipt, store plates at 2 - 8°C.

Procedure:

Using a sterile 10µL inoculating loop or a swab, streak *E. coli* broth culture onto each sector of the plate. If streaking with a swab, remove excess broth against the side of the growth vessel prior to streaking.

Incubate overnight at 37°C.

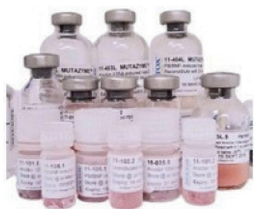
Expected Results:

Sector		Strain Response			
		Strain Name	**MOLTOX® Part Number**	**Strain Name**	**MOLTOX® Part Number**
Designation	**Description**	***E.coli* WP2**	**72-187L**	***E.* coli WP2 (pKM101)**	**72-002L**
		E.* coli WP2 *(uvrA)	**72-188L**	***E.coli* WP2 *(uvrA,* pKM101)**	**72-003L**
1 (I, A)	MGA	-		-	
2 (II, B)	MGA + Tryptophan	+		+	
3 (III, C)	MGA +Tryptophan/ Ampicillin	-		+	

MOLTOX®
Molecular Toxicology, Inc.

Product Information

S9 FAQs



What is the difference between S9 and lyophilized S9?

Lyophilized S9 is S9 that has been freeze-dried.
Lyophilized S9 can be stored in a standard freezer (approx. -20°C) unlike standard S9 which requires ultralow freezer storage (approx. -80°C).
Once reconstituted with ice-cold, sterile water, lyophilized S9 is equivalent to standard S9.

What is the difference between S9 and S9 Mix?

S9 is the supernatant from an organ homogenate, usually liver, that has been centrifuged at 9000g.
Microsomes in the supernatant contain cytochrome P450 metabolic enzymes.
S9 is often thus used to assess the mutagenic potential of chemical compounds.
S9 requires an NADPH-supported oxidation system to function in a test method.
Such system includes NADP, glucose-6-phosphate, $MgCl_2$, and KCl, in a phosphate buffer. These are often called "co-factors".
Once S9 is mixed with these co-factors it becomes "S9 Mix**"**.

What is the difference between S9 and Mutazyme™?

S9 is the supernatant from an organ homogenate, usually liver, that has been centrifuged at 9000g.
Microsomes in the supernatant contain cytochrome P450 metabolic enzymes.
S9 is often thus used to assess the mutagenic potential of chemical compounds.
Mutazyme"" is lyophilized (i.e., freeze-dried) S9 Mix**.**
Depending on part number, Mutazyme™ is available in S9 Mix concentrations of 5%, 10%, and 30%.
{See also "What is the difference between S9 and S9 Mix?**")**

What is the standard concentration of S9 Mix to use in my Ames Assay?

The most commonly used concentration is 10% followed by 5%.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

S9 (Post - mitochondrial Supernatant)

Intended Use: S9 is an exogenous mammalian source of cytochrome-P450 enzymes. When delivered to the test system in the presence of NADP and cofactors for NADPH-supported oxidation (i.e., S9 mix), P450-mediated metabolism of potential carcinogens may result in the generation of metabolites that exhibit activities that are not observed in the parental material.

Warnings and Precautions: For Laboratory Use.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, dispose of all materials according to location-specific biohazard procedures.

Storage: Upon receipt, store frozen S9 under ultra-low freezer conditions ([- 80°C] > [-70°C]). Store lyophilized S9 under standard freezer conditions (approximately- 20°C).
When thawing S9, do so under strict observation, preferably thawing S9 on ice. Thaw only what you expect to use that day; discard any unused S9. Do not refreeze as freeze/thaw cycles of S9 will affect the quality of the material and may cause erroneous results.

Reconstituting lyophilized S9: When reconstituting lyophilized S9, use ice-cold, sterile deionized water. Add a volume of water equivalent to the fill size in milliliters on the product vial. Store the reconstituted S9 on ice until use.

NADPH regenerating system: S9 requires an NADPH regenerating system, not included. S9 combined with an NADPH regenerating system is referred to as an S9 mix. Refer to appropriate test method for information on the appropriate system. MOLTOX® offers a prepared NADPH regenerating system (Regensys™ A and Regensys™ B) that, when mixed with S9, may be appropriate for your assay. MOLTOX® also offers a lyophilized complete S9 mix (Mutazyme™) that, when reconstituted, may also be appropriate for your assay.

Please contact MOLTOX® for further information.

Procedure: As S9 is used in several types of assays, there is no specific method that applies to all testing. Please refer to the following guidelines for information on how to use S9:

In vitro genotoxicity tests -

OECD 471 - Genetic Toxicology: Bacterial Reverse Mutation Assay

OECD 473 - Genetic Toxicology: *In vitro* Mammalian Cytogenetic Test

OECD 476 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests using the *Hprt* and *xprt* genes

OECD 487 - Genetic Toxicology: *In vitro* Mammalian Cell Micronucleus Test

OECD 490 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene

OECD guidelines can be obtained free of charge from www.oecd.org.

Expected Results: Refer to the appropriate OECD guideline for expected assay results.

Induction Information: PB/BNF-Phenobarbital/β-Naphthoflavone

Matsushima, et. al. 1976. In: de Serres, F.J, et. al., editors. *In Vitro Metabolic Activation in Mutagenesis Testing.* Amsterdam (NL) Elsevier/North Holland p. 85-88.

Additional Information: For additional information on the Bacterial Reverse Mutation Assay (i.e., the Ames Assay), refer to:

Maron, D. and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutat. Res., 133, 173 - 215.

Mortelmans, K. and E. Zeiger (2000) The Ames *Salmonella/* microsome mutagenicity assay, Mutat. Res., 455, 29 - 60.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Mutazyme™

Part Number	Description	Reconstituted Fill Size
11-404L	10% S9 Mix, PB/BNF induced	20 ml
11-405L	5% S9 Mix, PB/BNF induced	20 ml
11-406.3L	30% S9 Mix, PB/BNF induced	3.25 ml

Intended Use: Conveniently preformulated lyophilized S9 Mix. Once reconstituted and delivered to the test system, P450-mediated metabolism of potential carcinogens may result in the generation of metabolites that exhibit activities that are not observed in the parental material.

Warnings and Precautions: For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures.

After use, dispose of all materials according to your institutional biohazard program.

Storage: Upon receipt, store Mutazyme™ under standard freezer conditions (approximately - 20°C).

Reconstituting Mutazyme™: When reconstituting Mutazyme™, use ice-cold, sterile deionized water. Add a volume of water equivalent to the fill size in milliliters on the product vial. Store the reconstituted S9 Mix on ice until use.

Only reconstitute enough Mutazyme™ for use that day. Do not refreeze unused material as freeze/thaw cycles of S9 Mix will affect the quality of the material and may cause erroneous results.

Procedure: As S9 Mix is used in several types of assays, there is no specific method that applies to all testing. Please refer to the following guidelines for information on how to use S9 Mix:

In vitro genotoxicity tests-

OECD 471 - Genetic Toxicology: Bacterial Reverse Mutation Assay

OECD 473 - Genetic Toxicology: *In vitro* Mammalian Cytogenetic Test

OECD 476 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests using the *Hprt* and *xprt* genes

OECD 487 - Genetic Toxicology: *In vitro* Mammalian Cell Micronucleus Test

OECD 490 - Genetic Toxicology: *In vitro* Mammalian Cell Gene Mutation Tests using the Thymidine Kinase Gene

OECD guidelines can be obtained free of charge from www.oecd.org.

Expected Results: Refer to the appropriate OECD guideline for expected assay results.

Induction Information:
PB/BNF - Phenobarbital/β-Naphthoflavone

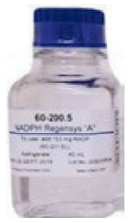
Matsushima, et. al. 1976. In: de Serres, F.J, et. al., editors. *in Vitro Metabolic Activation in Mutagenesis Testing.* Amsterdam {NL) Elsevier/North Holland p. 85-88.

Matsushima, et. al. 1976. In: de Serres, F.J, et. al., editors. *in Vitro Metabolic Activation in Mutagenesis Testing.* Amsterdam

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Regensys™ A (60-200)



Intended Use:

Conveniently preformulated, filter sterilized S9 mix components. Ready for use after addition of S9 and NADP (see 60-201; Regensys™ B) . Mixing of Regensys™ A, Regensys™ B, and S9 results in a cytochrome-based P450 metabolic oxidation System (i.e., "S9 mix").

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After preparation of S9 mix, dispose of all materials according to institutional biohazard procedures.

Storage:

Upon receipt, store Regensys™ A at 2 - 8°C.

Procedure:

Remove approximately 1 ml of Regensys™ A and add to the Regensys™ B vial to solubilize the NADP. Mix thoroughly by repeat pipetting up and down or capping the vial and mixing mechanically or by hand. Remove the entire solubilized contents from the Regensys™ B vial and transfer back into the Regensys™ A bottle. Repeat above steps if desired.

Referring to the below table, add appropriate volume of S9 and ice-cold, sterile dH_20 to Regensys™ A/B to achieve desired S9 mix concentration. While the system supports S9 mix concentrations ranging from 4% - 10%, the most commonly used concentration is 10% followed by 5%. Cap and invert (3x) or swirl to mix gently. Place on ice.

For the Bacterial Reverse Mutation Test (i.e., "Ames assay", OECD471), use 0.5 ml of this mix per plate.

60-200.15/60-201.15L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	0.60	0.75	0.90	1.05	1.20	1.35	1.50
Ice-cold, sterile dH_2O		0.90	0.75	0.60	0.45	0.30	0.15	0.00
Total Volume		15						

60-200.4/60-201.4L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	1.60	2.00	2.40	2.80	3.20	3.60	4.00
Ice-cold, sterile dH_2O		2.40	2.00	1.60	1.20	0.80	0.40	0.00
Total Volume		40						

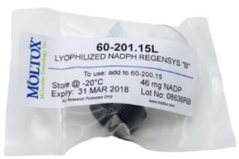
60-200.5/60-201.5L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	2.00	2.50	3.00	3.50	4.00	4.50	5.00
Ice-cold, sterile dH_2O		3.00	2.50	2.00	1.50	1.00	0.50	0.00
Total Volume		50						

Expected Results: See your institute's SOP, historical data or the appropriate OECD guidelines for expected assay results.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Regensys™ B (60-201)



Intended Use:

Convenient, pre-weighed aliquot of lyophilized NADP. Add to Regensys™ A (see 60-200) to complete NADPH Regenerating system. Mixing of Regensys™ A, Regensys™ B, and S9 results in a cytochrome-based P450 metabolic oxidation system (i.e., "S9 mix").

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After preparation of S9 mix, dispose of all materials according to institutional biohazard procedures.

Storage:

Upon receipt, store Regensys™ B frozen at - 20°C or colder.

Procedure:

Remove approximately 1 ml of Regensys™ A and add to the Regensys™ B vial to solubilize the NADP. Mix thoroughly by repeat pipetting up and down or capping the vial and mixing mechanically or by hand. Remove the entire solubilized contents from the Regensys™ B vial and transfer back into the Regensys™ A bottle. Repeat above steps if desired.

Referring to the below table, add appropriate volume of S9 and ice-cold, sterile dH_20 to Regensys™ A/B to achieve desired S9 mix concentration. While the system supports S9 mix concentrations ranging from 4%- 10%, the most commonly used concentration is 10% followed by 5%. Cap and invert (3x) or swirl to mix gently. Place on ice.

For the Bacterial Reverse Mutation Test (i.e., "Ames assay", OECD471), use 0.5 ml of this mix per plate.

60-200.15/60-201.15L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	0.60	0.75	0.90	1.05	1.20	1.35	1.50
Ice-cold, sterile dH_2O		0.90	0.75	0.60	0.45	0.30	0.15	0.00
Total Volume		15						

60-200.4/60-201.4L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	1.60	2.00	2.40	2.80	3.20	3.60	4.00
Ice-cold, sterile dH_2O		2.40	2.00	1.60	1.20	0.80	0.40	0.00
Total Volume		40						

60-200.5/60-201.5L		Concentration						
		4%	5%	6%	7%	8%	9%	10%
S9	mls	2.00	2.50	3.00	3.50	4.00	4.50	5.00
Ice-cold, sterile dH_2O		3.00	2.50	2.00	1.50	1.00	0.50	0.00
Total Volume		50						

Expected Results: See your institute's SOP, historical data or the appropriate OECD guidelines for expected assay results.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Regensys™A Plus (60-250)

Intended Use:

Conveniently preformulated, filter sterilized S9 mix components. Ready for use after addition of S9 and NADP (see 60-251 Regensys™ B Plus). Mixing of Regensys™ A Plus, Regensys™ B Plus, and S9 results in a cytochrome-based P450 metabolic oxidation system (i.e., "S9 mix").

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After preparation of S9 mix, dispose of all materials according to institutional biohazard procedures.

Storage:

Upon receipt, store Regensys™ A Plus at 2- 8°C.

Procedure:

Remove approximately 1 ml of Regensys™ A Plus and add to the Regensys™ B Plus vial to solubilize the NADP. Mix thoroughly by repeat pipetting up and down or capping the vial and mixing mechanically or by hand. Remove the entire solubilized contents from the Regensys™ B Plus vial and transfer back into the Regensys™ A Plus bottle. Repeat above steps if desired.

Referring to the below table, add appropriate volume of S9 and ice-cold, sterile dH_20 to Regensys™ A/B Plus to achieve desired S9 mix concentration. The system supports S9 mix concentrations ranging from 5% - 30%. Cap and invert (3x) or swirl to mix gently. Place on ice.

For the Bacterial Reverse Mutation Test (i.e., "Ames assay", OECD471), use 0.5 ml of this mix per plate.

60-250.15/60-251.15L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	0.75	1.50	2.25	3.00	3.75	4.50
Ice-cold, sterile dH_2O		3.75	3.00	2.25	1.5	0.75	0
Total Volume		15					

60-250.4/60-251.4L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	2	4	6	8	10	12
Ice-cold, sterile dH_2O		10	8	6	4	2	0
Total Volume		40					

60-250.5/60-251.5L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	2.5	5	7.5	10	12.5	15
Ice-cold, sterile dH_2O		12.5	10	7.5	5	2.5	0
Total Volume		50					

Expected Results: See your institute's SOP, historical data or the appropriate OECD guidelines for expected assay results.

MOLTOX®
Molecular Toxicology, Inc.

Product Information

Regensys™ B Plus (60-251)

Intended Use:

Convenient, pre-weighed aliquot of lyophilized NADP. Add to Regensys A Plus (see 60-250) to complete NADPH Regenerating system. Mixing of Regensys A Plus, Regensys B Plus, and S9 results in a cytochrome-based P450 Metabolic oxidation System (i.e., "S9 mix").

Warnings and Precautions:

For Laboratory Use Only.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After preparation of S9 mix, dispose of all materials according to institutional biohazard procedures.

Storage:

Upon receipt, store Regensys B Plus frozen at - 20°C or colder.

Procedure:

Remove approximately 1 ml of Regensys A Plus and add to the Regensys B Plus vial to solubilize the NADP. Mix thoroughly by repeat pipetting up and down or capping the vial and mixing mechanically or by hand. Remove the entire solubilized contents from the Regensys B Plus vial and transfer back into the Regensys A Plus bottle. Repeat above steps if desired.

Referring to the below table, add appropriate volume of S9 and ice-cold, sterile dH_20 to Regensys A/B Plus to achieve desired S9 mix concentration. The system supports S9 mix concentrations ranging from 5%- 30%. Cap and invert (3x) or swirl to mix gently. Place on ice.

For the Bacterial Reverse Mutation Test (i.e., "Ames assay", OECD471), use 0.5 ml of this mix per plate.

60-250.15/60-251.15L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	0.75	1.50	2.25	3.00	3.75	4.50
Ice-cold, sterile dH_2O		3.75	3.00	2.25	1.5	0.75	0
Total Volume		15					

60-250.4/60-251.4L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	2	4	6	8	10	12
Ice-cold, sterile dH_2O		10	8	6	4	2	0
Total Volume		40					

60-250.5/60-251.5L		Concentration					
		5%	10%	15%	20%	25%	30%
S9	mls	2.5	5	7.5	10	12.5	15
Ice-cold, sterile dH_2O		12.5	10	7.5	5	2.5	0
Total Volume		50					

Expected Results: See your institute's SOP, historical data or the appropriate OECD guidelines for expected assay results.

Citations

Ames Assay

Maron, D.M, and B.N. Ames (1983) Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113, 173 – 213.

Green, M.H.L, and W. J. Muriel (1976) Mutagen testing using trp+ reversion in Escherichia coli, Mutat. Res. 38, 3 – 31.

Mortelmans, K., and E. Riccio (2000) The bacterial tryptophan reverse mutation assay with Escherichia coli WP2, Mutat. Res. 455, 61 – 69.

OECD. 2000. Guideline for Testing of Chemicals, Genetic Toxicology No. 471, Organisation for Economic Co-Operation and Development, Paris, 26 June 20.

Matsushima, et al., In Vitro Metabolic Activation in Mutagenesis Testing (F.J. de Serres, ed.), Elsevier, 1976, p 85.

Miniscreen Ames Assay

Brooks, T.M. (1995) The use of a streamlined bacterial mutagenicity assay, the miniscreen, Mutagenesis, 10, 441-448.

Diehl, M.S., S.L. Willaby, and R.D. Snyder (2000) Comparison of the results of a modified miniscreen and the standard bacterial reverse mutation assays. Environ. Mol. Mutagen, 36, 72 – 77.

Escobar, P.A, R. A. Kemper, J. Tarca, J. Nicolette, M. Kenyon, S. Glowienke, et al. (2013) Bacterial mutagenicity screening in the pharmaceutical industry. Mutat. Res. 578 (1-2), 210-224.

Flamand, N., J. R. Meunier, P. A. Meunier, and C. Agapakis-Caussö (2001) Mini mutagenicity test: a miniaturized version of the Ames test used in a prescreening assay for point mutagenesis assessment. Toxicol. in Vitro, 15, 105-114.

Fluctuation Assay

Bridges, B.A. (1980) The fluctuation test. Arch Toxicol. 46 (1 -2), 41 – 44.

Gatehouse, D.G., and G. F. Delow (1979) The development of a "microtitre®" fluctuation test for the detection of indirect mutagens, and its use in the evaluation of mixed enzyme induction of the liver. Mutat. Res. 60(3), 239 – 252.

Green, M. H., W. J. Muriel, and B. A. Bridges (1976) Use of a simplified fluctuation test to detect low levels of mutagens. Mutat. Res. 38(1), 33 – 42.

Hubbard, S.A., M. H. L. Green, D. Gatehouse, and J. W. Bridges (1984) The fluctuation test in bacteria, in: B. J. Kilbey, M. Legator, W. Nichols, and C. Ramel (Eds.), Handbook of mutagenicity test procedures, 2nd ed. Elsevier, pp 141 – 160.

microAmes Assay

Escobar, P.A, R.A. Kemper, J. Tarca, J. Nicolette, M. Kenyon, S. Glowienke, et al. (2013) Bacterial mutagenicity screening in the pharmaceutical industry. Mutat. Res. 578, 210-224.

Pant, K., S. Bruce, J. Sly, M.K. Laforce, S. Springer, M. Cecil, E. Andrus, E. Dakoulas, V.O. Wagner, N.J. Hewitt, et al. (2016) Bacterial mutagenicity assays: Vehicle and positive control results from the standard Ames assay, the 6- and 24-well miniaturized plate incorporation assays and the Ames II™ Assay. Environ. Mol Mutagen. 57, 483-496.

Proudlock, R., and K. Evans (2016) The micro-Ames test: A direct comparison of the performance and sensitivities of the standard and 24-well plate versions of the bacterial mutation test. Environ. Mol. Mutagen. 57, 687- 705.

S9 Characterization

Evans, K., S. Boinnotte, E. Zeiger, J. Cheung, and A. Lynch (2025) A comparative analysis of select P450 enzymes in uninduced and PB/BNF-induced hamster and rat liver S9. Muta. Res. Genet. Toxicol. Environ. Mutagen. 902.

MOLTOX®

Molecular Toxicology, Inc.

157 Industrial Park Drive Boone, NC 28607 828.264.9099 Toll Free: 800.536.7232 Fax: 828.264.0103

www.MOLTOX.com sales@moltox.com

2026