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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.

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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** (1 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 mls 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).

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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

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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 Dimethylbenzanthracene[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
TA100		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[d]	5
	OR	Cyclophosphamide	100
	OR	Benzopyrene	20
	OR	7,12 Dimethylbenzanthracene[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		2-Aminofluorene[a]	20
	OR	2-Aminoanthracene[a]	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