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Molecular Toxicology, Inc.

Phenotype Test Kit Instruction Manual

31-600

INTRODUCTION

Phenotype tests are performed to verify the genotype of *S. typhimurium* and *E. coli* strains utilized in the reverse bacterial mutation test. This is a requirement of the OECD 471 guideline. As opposed to traditional tests that require multiple plates per strain and specialized equipment, this kit allows for use of one plate per strain, can be used with both *S. typhimurium* and *E. coli* tester strains, and utilizes Mitomycin C discs in place of UV light exposure. Results are available the next day.

Principle

Wells 1 – 4 contain Minimal Glucose Agar (MGA) with excess histidine, biotin, and tryptophan. The strain culture is added to each well and a specific phenotype test disc applied to each. Discs and the characteristic they detect are as follows:

Designation	Disc type	Detects	Genotype
C	Crystal Violet	Sensitivity to crystal violet	*rfa* deep mutation
A	Ampicillin	Ampicillin resistance	+/- pKM101 R-factor plasmid
T	Tetracycline	Tetracycline resistance	+/- pAQ1 plasmid
M	Mitomycin C	Sensitivity to mitomycin C [1]	*uvrA* and *uvrB* repair deficiency [1]

[1] Traditionally, *uvrA* and *uvrB* repair deficiency is tested via exposure to UV light, a cross-linking agent. Use of mitomycin C, also a cross-linking agent, is used in place of UV exposure.

Wells 5 and 6 contain MGA with excess histidine and biotin and MGA with excess tryptophan and biotin, respectively. These wells test for tryptophan and histidine growth requirements.

Procedure – proceed as below for each strain using sterile technique

1. Mix 100 µL of bacterial suspension culture with 4 mL molten top agar at 45°C.
2. Dispense 0.5 mL of the mix into each well of the 6-well phenotype plate ensuring it covers the surface of each well. Leave the plate on a level surface for the agar to gel.
3. Place one C, A, T and M (crystal violet, ampicillin, tetracycline and mitomycin C) disc near the center of wells 1 to 4 respectively.
4. Incubate the plates at 37°C for 16-24 hours.
5. Hold the plate 3 or 4 cm above a printed page then estimate the diameter of the clear zone of inhibition in wells 1 to 4 using a ruler. Record density of growth in wells 5 and 6.

Expected results	Zone of inhibition mm				Growth	
Strain	C	A	T	M	trp$^-$	his$^-$
TA1535, TA1537, TA1538	X	X	X	X	++	-
TA97a, TA98, TA100	X		X	X	++	-
TA102	X				++	-
WP2		X	X		-/+	++
WP2 *uvrA*		X	X	X	-/+	++
WP2 pKM101			X		-/+	++
WP2 *uvrA* pKM101			X	X	-/+	++

X	zone > 10 mm			
-	no growth	-/+	no or weak growth	
+	weak growth	++	strong growth	

Version

04.07.16

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