AMES TEST: Bacterial Reverse Mutation Assay

1. Introduction

The bacteria reversed mutation assay (Ames Test) is used to evaluate the mutagenic properties of test articles. The test uses amino acid-dependent strains of *S. typhimurium* and *E.coli*. In the absence of an external histidine source, the cells cannot grow to form colonies. Colony growth is resumed if a reversion of the mutation occurs, allowing the production of histidine to be resumed. Spontaneous reversions occur with each of the strains; mutagenic compounds cause an increase in the number of revertant colonies relative to the background level.

2. Principal of test method

Bacterial culture medium is inoculated with the appropriate *Salmonella* or *E. coli* strain and incubated overnight. A dose rangefinder for the test chemical is carried out using strain TA100 only over a wide dose of range. Bacterial culture, test chemical and S9 mix are incubated for one hour. Thereafter the incubation solution is mixed with soft agar and added to minimal agar plates. The plates are incubated for 48 – 72 hours. After this time the numbers of revertant colonies are counted.

3. Tester Strains

Characteristics of Tester Strains

All *Salmonella* strains are histidine-, the used *E. coli* strain tryptophan dependent. Revertants are identified as colonies that grow in low levels of histidine or tryptophan. Frameshift and base-pair substitution defects are represented to identify of both

types. Additional genetic markers serve to make the strains more sensitive to certain types of mutagens.

A list of these additional genetic markers and strain characteristics are shown in Table 1.

***S. typhimurium* strain**	**Gene Affected**	**DNA-repair**	**LPS**	**Biotin Requirement**	**Plasmids**	**Mutational Event**
S. typh. TA98	*hisD*	*uvrB*	*rfa*	*bio-*	pKM101	frameshift
S. typh. TA100	*hisG*	*uvrB*	*rfa*	*bio-*	pKM101	base-pair substitution
S. typh. TA102	*hisG*	-	*rfa*	*bio-*	pKM101, pAQ1	base-pair substitution
S. typh. TA1535	*hisG*	*uvrB*	*rfa*	*bio-*	-	base-pair substitution
S. typh. TA1537	*hisC*	*uvrB*	*rfa*	*bio-*	-	frameshift
E. coli WP2 uvrA	*trp*	*uvrA*	-	-	-	base-pair substitution

Table 1: Characteristics of *Salmonella* and *E. coli* strains used for the Ames Test

The DNA repair mutation (*uvrA/B*) eliminates excision repair, a repair pathway for DNA damage from UV light and certain mutagens. The presence of the *uvrA/B* mutation makes the strains more sensitive to the test articles that induce damage in this manner. The *uvrA/B* mutation is part of a deletion mutation extending into a gene for biotin synthesis; therefore, the biotin requirement is a result of the deletion of this region. The *uvrA/B* mutation is indicated by sensitivity to UV light.

The *rfa* mutation changes the properties of the bacterial cell wall and results in the partial loss of the lipopolysaccharide (LPS) barrier increasing permeability of cells to certain types of chemicals. The *rf*a mutation is indicated by sensitivity to crystal violet.

The R factor plasmid (pKM101) makes the strains more responsive to a variety of mutagens. The plasmid carries an ampicillin resistance gene; therefore ampicillin resistance indicates that the strains retain the plasmid. The pAQ1 plasmid carries a tetracycline resistance gene, therefore tetracycline resistance indicates that strain TA102 retains the plasmid.

4. Exposure concentrations

The maximum test item concentration for non cytotoxic substances is 5 mg/plate or 5 µl/plate, but depends on solubility.

5. S9 Mix(Metabolic Activation)

Aroclor^{TM} 1254-induced rat liver S9, prepared from adult Sprague Dawley rats, is supplemented with different cofactors (glucose-6-phosphate, NADP-Na_2) to a final protein concentration of 2 mg/ml incubation mixture.

6. Controls

Strains are tested with the solvent or vehicle as the negative control and with known mutagens to demonstrate that the assay is working efficiently (positive control) and also to demonstrate that the metabolic activation system is operating.

positive controls	w/o metabolic activation	w metabolic acitvation
	2-nitrofluorene) sodium azide mitomycin C 9-aminoacridine methyl methanesulfonate	2-aminofluorene 1,8-dihydroxyanthrachinone 2-aminoanthracene cyclophosphamide

7. Evaluation/Analysis

Besides cytotoxicity, precipitation and viability the number of revertant colonies per plate is determined. The mutant frequency is expressed as the quotient of the number of revertant colonies over the number of colonies in the negative control.

8. Interpretation of Results

A mutagenic potential of a test item is assumed if the mutant frequency is 2.0 or higher. A dose effect relationship could underline this conclusion.
A possible mutagenic potential is assumed if the quotient ranges between 1.7 to 1.9 in combination with a dose effect relationship.
No mutagenic potential is assumed if all quotients range between 1.0 (or lower) to 1.6. A nonexistent dose effect relationship could underline this conclusion.

9. References:

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