

MUTAGENIC ASSAY

BACTERIAL REVERSE MUTATION – AMES II TEST

This standard AMES test, based on bacterial reverse mutation, is commonly used for initial screening to detect point mutations induced by lead drugs or test compounds in *Salmonella typhimurium* bacteria in *in vitro* system. Bacteria cultures are exposed to test compounds in the presence and the absence of exogenous metabolic activation enzyme system. After incubation time, positive bacterial revertants are counted and compared to the number of spontaneous positive bacterial revertants in vehicle control wells in comparison to positive mutagenic compounds.

Format of test compounds: in solution prepared by the customer

Quantity of sample required: 1ml of a stock solution at 125mg/ml dissolved in aqueous solution or in 100% of DMSO for poorly-soluble test compound.

Turnaround time to deliver the report: 15 days upon signature of a “R&D Services Contract”

Experimental design :

- AMES II™ kit using microplates of 384 wells. Conditions are performed in triplicates
- Approximately 10^7 *his*-bacteria are exposed to various conditions. Two strains are tested: one is *S. typhimurium* TA98 and an equimolar mixture of six strains *S. typhimurium* TA7001 to TA7006 for the detection of frame-shift and specific base-pair substitution mutations, respectively
- Dose-response curve with 6 concentrations (recommended top dose at 5000 µg/ml and serial dilution at 5-fold intervals) of test compound
- Metabolic activation with and without S9 hepatic (rat) fraction
- Use organic solvent DMSO for poorly-soluble test compounds to get a final concentration below 4 % in presence of bacteria

Control drugs :

- 2-nitrofluorene (2-NF) without S9 fraction
- 4-nitroquinoline N-oxide (4-NQO) without S9 fraction
- 2-aminoanthracene (2-AA) with S9 fraction
- Spontaneous condition (vehicle-treated condition)



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Analysis :

- Detection is performed by colorimetric detection and quantification of positive wells is completed visually. This method is based on method reported by these following groups: Gee, Maron and Ames (1994) and Flückiger-Isler *et al.* (2004).

Report document :

- Information included in the report is adapted upon customer's request.

Typical results with control drugs:

Bacterial strains	Positive and negative controls	Total number of positive bacterial revertants per condition
Mixture of TA7001-TA7006	Vehicle	≤ 5
	4-NQO (500ng/ml)	≥ 25
	2-AA (5µg/ml) + S9	≥ 15
TA98	Vehicle	≤ 8
	2-NF (2µg/ml)	≥ 25
	2-AA (5µg/ml) + S9	≥ 25

A fold increase bigger than 2 of the total number of positive bacterial revertants induced by the exposition with test compounds over the total number of positive bacterial revertants in spontaneous (vehicle) condition represent mutagenic compound.

Cost :

- Please inquire to CRBM

References: 1) Gee, Maron and Ames (1994) Detection and classification of mutagens: A set of base-specific Salmonella tester strains. Proc Nat Acad Sci USA 91 p11606-11610.
2) Flückiger-Isler *et al.* (2004) Assessment of the performance of the Ames II assay: A collaborative study with 19 coded compounds. Mutation Res 558, p181-197.