BACTERIAL REVERSE MUTATION TEST (AMES)

The Bacterial Reverse Mutation test assesses the mutagenic potential of a compound and is commonly employed as an initial **screen for genotoxic activity** and, in particular, for mutation-inducing activity, which involve substitution, addition or deletion of one of the DNA base pairs.

STRAINS

Ames testing uses several strains of the bacterium Salmonella typhimurium which carry a defective (mutant) gene that renders them unable to synthesize the amino acid histidine. The Ames test investigates the potential of the test compound to result in a **back mutation** that causes the gene to regain its function and grow in a histidine-free medium.

At least five strains of bacteria should be used :

- **Base-pair substitution**: S. typhimurium TA1535 and TA100 and TA102 or E. coli WP2 uvrA or E. coli WP2 uvrA (pKM101)
- **Frame-shift**: S. typhimurium TA1537 or TA97 or TA97a and TA98: frame-shift

METABOLIZATION

Mutagenic potential can be investigated in the Ames test in the presence or absence of a **metabolizing system** (rat liver S9 fraction) to identify pro-mutagens as well as directly acting mutagens.

CONDITIONS

The standard methods apply for most pure chemicals and consist of **incorporation method, preincubation method, fluctuation method and suspension method**. An additional method referred as **Treat and Wash method**, applies for proteins natural or synthetized, mixture, plant extract, antibiotics, or cytotoxic compound (oncologic).

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Optimized genotoxicity characterization for limited sample quantities

For **non GLP exploratory screening** S. Typhimurium TA98 (frameshift mutation) and TA100 (base-pair substitution) are two common strains used for this reduced Ames test since they both have rfa mutations (a defective lipopolysaccharide layer that makes bacteria more permeable to larger molecules), uvrB mutations (elimination of excision repair of DNA damage) and pKM101 plasmid (increasing error-prone repair of DNA damage).

GenEvolutioN has developed **High Throughput miniaturized conditions (non GLP)** when a limited amount of sample is available (discovery genotoxicity screening and impurity genotoxicity characterization), such as **MINI Ames test or NANO® Ames test**.

Test model	Salmonella typhimurium TA 98, TA100, TA1535, TA1537, TA102 (optional E.coli WP2 UvrA)
Assay Controls	Negative control: Aqueous and organic solvents Positive control: Strain-specific positive control
Exposure time	48 – 72 hours
Test item quantity	Standard condition (incorporation or preincubation): 1 g Treat & Wash conditions: 250 mg **MINI Ames, 25 wells plate (agar): 250 mg** **MINI Ames, MPF 384 wells plate (liquid): 65 mg** **NANO® Ames (25 and 96 wells plate): 35 µg**, also applicable for unknown impurities in solution (no additional synthesis of compound)
Endpoint	Growth of revertant colonies combined with genomic sequencing for NANO® Ames
Data delivery	Mean number of revertant colonies per plate • 2-fold increase with vehicle control (TA 98, TA 100, TA97, TA97a, TA102, and WP2 UvrA) • 3-fold increase with vehicle control (TA 1535 and TA 1537)
Timeline	2 – 3 weeks
Regulatory statut	GLP compliance for Standard and Treat & Wash conditions with Formulation Analysis (optional)