

## Bacterial reverse mutation test (Ames test)

**Background:** The bacterial reverse mutation test (also called Ames test) is used to detect point mutations, which involves substitution, insertion or deletion of one or a few DNA base pairs. The test is performed according to the OECD Guidelines for Testing of Chemicals, Test No. 471 (adopted in 1997) and employs auxotrophic strains of *Salmonella typhimurium* and *Escherichia coli*. Point mutations were made in the histidine (*S.typhimurium*) or the tryptophan (*E.coli*) operon, thus blocking bacterial biosynthesis of the corresponding amino acids. A chemical's mutagenic potential is evaluated by detecting the appearance of the reverse mutants of the auxotrophic strains, making them prototrophs, able to grow in corresponding deficient media. This test is commonly used as a quick screen to estimate the mutagenic potential of new chemicals and drug candidates. Modified Ames test can also be performed upon request. It includes addition of chemically induced rat liver S9 fraction to simulate the effect of metabolism, since certain compounds, like benzopyrene, become mutagenic only after their metabolic conversion.

**Service Details:** Six bacterial strains (2 of them as a mixture) are used according to the OECD Guidelines for Testing of Chemicals: *S.typhimurium* TA98, TA100, TA1535, TA1537, and *E.coli* wp2[pKM101] + wp2 uvrA mixed 1:2. Chemicals are tested according to the traditional Ames test using standard incorporation protocol (Revised methods for the Salmonella mutagenicity test. Maron D.M., Ames B.N.//Mutat. Res. 1983, 113 (3-4):173-215). Three concentrations of the test agent as well as a positive and negative controls are tested using 6 tester strains (two are mixed). The colonies are counted and the results are expressed as the number of revertant colonies per plate. Compounds with known mutagenic activity are used for positive control for each tester strain: TA98 - 2-nitrofluorene (0.4 µg/ml); TA100 - 4-nitroquinoline N-oxide (0.04 µg/ml); TA1535 - NaN<sub>3</sub> (0.2 µg/ml); TA1537 - 9-aminoacridine (3 µg/ml); *E.coli* strains – 4-nitroquinoline N-oxide (0.04 µg/ml). For metabolic activation testing (S9), 2-aminoanthracene (0.4 µg/ml) is used for all strains. DMSO is used as a negative control.

**Deliverable:** Final report comprising information on the analysis, methodology, raw data, and interpretation of the results is provided.

**Sample Submission:** Approximately 80 µmol of dry compound (or equivalent in stock solution) is necessary for this test. Exact amount depends on the selected testing range and/or solubility of the test article.