

Mutagenicity Screening in Liquid Microplate Format using *Salmonella typhimurium* and *Escherichia coli* Strains (“Micro Ames Assay”)

Guideline:	OECD 471 (adopted 21 st July, 1997);
Objective:	To establish the potential of the test item to induce gene mutations in bacteria (<i>Salmonella typhimurium</i> and <i>Escherichia coli</i>) by means of reversions from auxotrophic strains to prototroph.
Tester strains:	<i>Salmonella typhimurium</i> TA98, TA1537 (for frameshift mutations); TA100, TA1535 and <i>Escherichia coli</i> WP2 <i>uvrA</i> (for base-pair substitution).
Metabolic activation:	The experiments are performed in the presence and absence of a post mitochondrial supernatant (S9) prepared from livers of phenobarbital/ β -naphthoflavone-induced rats.
Dose levels:	<u>In this screening test each test item dose and each control is investigated in 50 wells of a 96-well plate.</u> Maximum dose level: 5 mg/mL or 5 μ L/mL. One test is performed with at least ten dose levels (according to a geometric series) approximately half log or smaller intervals.
Controls:	Blank, untreated, strain specific positive (diagnostic mutagens) negative (vehicle) controls.
Procedure:	Bacteria are exposed to the concentration series of the test item, as well as positive and negative controls in the presence and absence of a post mitochondrial supernatant (S9) for 90 minutes in medium containing sufficient histidine (or tryptophan for <i>E. coli</i>) to support a basic bacterial growth (approximately two cell divisions) and aliquoted into 96-well plates. After 90 minutes, the exposure cultures are diluted with pH indicator medium lacking histidine (or tryptophan). The evaluation is performed after 48 and 72 hours exposure. The cells which undergone the reversion to prototrophy will grow into colonies. Metabolism by the bacterial colonies lowers the pH of the medium, changing the colour of the medium from purple to yellow. This colour change can be detected visually. The wells containing revertant colonies are counted for each dose and compared to a zero-dose (vehicle) control. In this screening test each test item dose and each control is investigated in 50 wells of a 96-well plate. A dose-related and/or statistically significant increase in the number of revertant colonies upon exposure to a test chemical relative to the zero-dose controls indicates that the chemical is mutagenic in the Micro Ames Assay.
Amount of test item required:	At least 65 mg
Draft Report:	Approximately 4 weeks from the arrival of the test item