In Vitro Genotoxicity Assays

Need

Genotoxicity is a required component of investigational new drug (IND) applications as well as an integral part of safety assessments for other product classes. Genotoxic compounds induce DNA damage though several modes of action, including deletions, single or double-stranded breaks. For new APIs, ingredients and formulations, determining their mutagenic potential is crucial to ensure the compound does not induce DNA damage, which could lead to cell death, cancer, or other adverse conditions. In addition, these genotoxicity tests are required by both the EPA, FDA, ECHA, and other regulatory bodies before bringing a drug or product to market.

Solution

LifeNet Health LifeSciences performs the Ames bacterial reverse mutation test and the *in vitro* micronucleus test in accordance with OECD guidelines. The genotoxic potential of a compound or chemical can be assessed early in the product development cycle to determine their relative risks, or later in the IND-enabling stage of drug development as it is a requirement for all IND submissions. The Ames bacterial reverse mutation test is based on the fluctuation method. This test uses essential amino acid-requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations; mutations which revert mutations present in the test strains and result in a restored ability of the bacteria to synthesize the required, essential amino acid. Bacteria are exposed to 6 concentrations of test article(s), as well as a positive and a negative control, for 90 minutes in medium containing enough histidine (*S. typhimurium*) or tryptophan (*E. coli*) to support approximately two cell divisions. The exposures are performed in the presence and absence of metabolic activation, provided by a liver homogenate, S9. After exposure, the cultures in each condition (negative control, test samples and positive controls) are diluted in pH indicator medium lacking histidine or tryptophan and aliquoted into the wells of a 384-well plate. After 2 days of incubation, bacterial cells that have undergone reversion to amino acid prototrophy will grow, reducing the pH of the medium resulting in a color change. The number of wells containing revertant colonies is counted for each dose and compared to a solvent (negative) control. This test can be performed as a full assessment (five strains) or as a screen (two strains).

The second test for genotoxicity is the *in vitro* mammalian micronucleus test. Chemicals or compounds may generate acentric chromosomes (clastogenic event) or inappropriately segregate whole chromosomes (aneugenic event) leading to genetic damage in the form of an erratic, smaller nucleus called micronucleus. Mammalian cells (i.e., CHO cells, TK-6 cells) are exposed to 6 concentrations of test article(s), as well as a positive and a negative control, for 1.5 – 2 population doublings. The exposures are performed in the presence and absence of metabolic activation, provided by a liver homogenate, S9. After exposure, the cells are harvested, the DNA is stained, and the plates are analyzed using fluorescence microscopy. The images are analyzed using image analysis software to identify the presence of micronuclei.

Accurate & reliable data

Fast turnaround times

Unsurpassed expertise

Collaborative approach

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Standard Protocol for Ames

ASSAY PARAMETERS	PROTOCOL
Model	*S. typhimurium* strains (TA98, TA100, TA1535 and TA1537) and *E. coli* strain WP2 uvrA(pKM101)
Replicates	3
Solvent of Choice	DMSO (preferred) or sterile water
Test Article Formulation	1000, 100, 10, 1, 0.1, and 0.01 µg/mL (depending on solubility)
Solvent Controls	DMSO, sterile water, or assay buffer
Positive Controls (without metabolic activation)	TA98 - 2-Nitrofluorene, TA100 and WP2 uvrA (pKM101) - 4-Nitroquinoline-N-oxide, TA1535 - N4-Aminocytidine, TA1537 - 9-Aminoacridine
Positive Controls (with metabolic activation)	TA98, TA100, TA1535, and TA1537 - 2-Aminoanthracene, WP2 uvrA (pKM101) - 2-Aminofluorene
Exposure Time	48 hours
Time to Complete	3-4 weeks from initiation
Regulatory	Non-GLP or GLP
Deliverables	Full Report and categorization (positive or negative for mutagenicity)

Standard Protocol for *In Vitro* Mammalian Micronucleus

ASSAY PARAMETERS	PROTOCOL
Model	TK-6 cells or CHO cells
Replicates	3
Solvent of Choice	DMSO (preferred) or sterile water
Test Article Formulation	1000, 100, 10, 1, 0.1, 0.01, and 0.001 µM (depending on solubility)
Solvent Controls	DMSO (preferred) or sterile water
Positive Controls (without metabolic activation)	Vinblastine and 4-Nitroquinoline-N-oxide
Positive Controls (with metabolic activation)	Benzo[a]pyrene
Exposure Time	1.5 – 2 population doublings
Time to Complete	4-6 weeks from initiation
Regulatory	Non-GLP or GLP
Deliverables	Full Report and categorization (positive or negative for mutagenicity)

Key References

OECD (2020), Test No. 471: Bacterial Reverse Mutation Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264071247-en.

OECD (2023), Test No. 487: In Vitro Mammalian Cell Micronucleus Test, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris, https://doi.org/10.1787/9789264264861-en.

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