BioReliance

Toxicology Services

TK6 Micronucleus Assay

Micronucleus Assay

Candidate compounds must be assessed for the potential to cause chromosomal damage early in the drug development process. One assay that is capable of detecting this type of damage is the Micronucleus assay. It detects small, extra nuclei in the cell cytoplasm that are indicative of chromosome fragments or whole chromosomes excluded from nuclei at cell division due to chemically induced damage. The micronucleus assay demonstrates high concordance with chromosome aberration analysis, but it is a faster test that requires less technical expertise. These desirable traits have led to its widespread use as an efficient and relatively simple method to screen small molecule drug candidates and other chemicals for clastogenic and aneugenic potential.

Resolution to a Problem

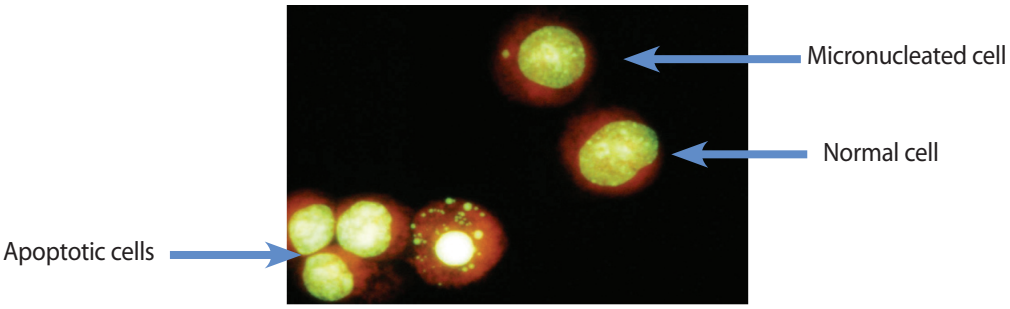
In vitro genotoxicity assays are usually conducted using rodent cell lines or human peripheral blood. Unfortunately, these assays often demonstrate a high rate of positive results. This is especially concerning when this same high rate is not seen in in vivo genotoxicity or rodent carcinogenicity data, thus implying that the in vitro tests are not predictive of in vivo genotoxicity or carcinogenicity studies. To evaluate this problem, recent studies compared rodent cell lines used in in vitro genotoxicity assays with p53-competent and human-derived cell lines.1, 2 Results show that the rodent cell lines are more likely to provide misleading positive results. The outcome from these initiatives, including the International Working Group on Genotoxicity (IWGT), is to use p53-proficient and human cells for in vitro genotoxicity assays such as micronucleus and chromosome aberration.

The TK6 Micronucleus (MN) Assay

TK6 cells are derived from a human B lymphoblastoid cell line. Therefore, they are p53 proficient and karyotypically stable. The TK6 Micronucleus assay provides a reduction in the percentage of non-relevant positive results compared to p53-mutated cell lines. Use of the TK6 Micronucleus (MN) assay eliminates donor variability that is seen in a MN assay with human peripheral blood lymphocytes (HPBL).

Scoring of Assay

View of TK6 Cells under fluorescence microscope after micronucleus assay
(Normal, apoptotic, and micronucleated mononucleate TK6 cells)



Fully Validated from Approved Guidelines with Regulatory Acceptance

- OECD Test Guideline 487 (approved July 2010)
- Fulfills Option 1 of ICH S2(R1) as an in vitro mammalian cell assay

Why BioReliance and TK6 MN

- Focus on Genetic Toxicology
- Rapid TAT
- p53-competent
- Human origin
- Less donor variability
- Explore MOA

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TK6 Micronucleus Assay

BioReliance's TK6 MN Assay

BioReliance uses TK6 cells from certified sources that are maintained in a master stock not greater than 24 passages and with population doubling at 1.3 % to 1.8 %. BioReliance offers an assay validated according to international guidance and industry-recognized standards. The assay includes multiple positive controls.

Options for this assay include a full GLP assay (in accordance with OECD guidelines and validated designs), a GLP version without a dose-range finder, and a non-GLP screening version

Follow Up to a Positive Result

The TK6 Micronucleus Assay is ideal for use as a follow up assay to explore mechanism of action (aneugenic or clastogenic) from an initial positive genotoxicity result. In a TK6 MN assay either FISH or CREST techniques may be used without the need to interrogate additional cells beyond the basic assay design.

BioReliance offers the industry's broadest range of in vitro and in vivo assays to assess the genotoxic potential of a drug candidate or chemical entity. These range from the AMES II assay to transgenic mouse models of carcinogenicity. All of our services are performed in accordance with international regulatory guidelines where appropriate. (Many are available as non-regulated screening assays as well.) Please contact a BioReliance Toxicology Consultant to prepare a testing plan that will best suit your needs.

Ordering Information

IN VITRO CYTOGENETICS							
	Protocol Number	**Assay Format**	**GXP**	**Test System**	**Assay Design**	**TAT - Weeks to Verbal**	**Material Required**
Micronucleus Assay	361.BTL	In Vitro with Microscopic scoring	GLP	TK6 Cells	Preliminary toxicity assay with 9 doses in single culture, 3-6 hours treatment +/-S9 with 23-40 hours recovery and 27 hour long-term treatment, 27 hour collection, score up to 4 dose levels for micronucleus induction, score under fluorescence microscope	6 weeks	200 mg

References

Fowler, et al, "Reduction of misleading ("false") positive results in mammalian cell genotoxicity assays. I. Choice of cell type." *Mutation Research* 742, 11-25 (2012)

Pfuhler, et al, "In vitro genotoxicity test approaches with better predictivity: Summary of an IWGT workshop" *Mutation Research* 723, 101-107 (2012)

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