HPRT-Forward-Mutation-Assay

1. Introduction

The HPRT assay is an *in vitro* mammalian cell gene mutation test. This test system is appropriate for use in the initial assessment of the genotoxicity of a test article.
V79 Chinese hamster cells have one functional copy of the gene which codes for the HPRT enzyme. HPRT enzyme activity is important for DNA synthesis. The use of the toxic nucleoside analog 6-thioguanine (6-TG) forms the basis for cell selection following treatment. Cells without a mutation are poisoned by 6-TG, while mutant cells survive and form colonies. Those cells that are able to form colonies are assumed to be mutant cells resulting from either a spontaneous mutation or from an induced mutation caused by a chemical agent.
This assay is used to evaluate the potential of a chemical, formulation or extract to induce mutations at the *hgprt* locus of CHO cells.

2. Principle of test method

Cells deficient in HPRT are selected by resistance to 6-thioguanine (TG). These cells are exposed to the test substance, both with and without metabolic activation, for 2-4 h or 24-72 h and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. Cytotoxicity is determined my measuring the relative cloning efficiency (survival) of the cultures after the treatment period. The treated cultures are maintained in growth medium for 3 days, characteristic of each selected locus and type, to allow near optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable

incubation time (ca. 10 days), colonies are stained and counted. The mutant frequency is derived from the number of mutant colonies in selective medium and the number of colonies in non-selective medium.

3. Exposure concentrations

In consideration of solubility and cytotoxicity the highest test item concentrations are 10 mM, 5 mg/ml and 5 µl/ml. At least four analysable concentrations are tested.

4. Controls

Concurrent negative (solvent or vehicle) and positive controls both with and without metabolic activation are included in each experiment.

positive controls	w/o metabolic activation	w metabolic acitvation
	N-methyl-N'-nitro-N-notrosoguanidine (MNNG)	7,12-dimethylbenz[a]anthracene (DMBA)

5. Evaluation/Analysis

Besides cytotoxicity and viability the number of mutant colonies in selective medium and the number of colonies in non-selective medium is determined. The mutant frequency is expressed as number of mutant cells per number of surviving cells.

6. Interpretation of results

A result is classified as positive if there is a concentration-related or reproducible increase in mutant frequency observed.

Positive results for the HPRT-mutation assay indicate that the test substance induces gene mutations in the cultured cells used. A positive concentration-response, that is reproducible is most meaningful.
There is no requirement for verification of a clear positive response. Equivocal results are clarified by further testing using modified experimental conditions. Negative results need to be confirmed on a case by case basis.