OECD GUIDELINE FOR TESTING OF CHEMICALS

480

Adopted:
23 Oct 1986

"Genetic Toxicology: *Saccharomyces cerevisiae*, Gene Mutation Assay"

Effective Date of Deletion
2 April 2014

1. INTRODUCTORY INFORMATION

• Prerequisites

– Solid, liquid, vapour or gaseous test substance
– Chemical identification of test substance
– Purity (impurities) of test substance
– Solubility characteristics
– Melting point/boiling point
– pH (where appropriate)
– Vapour pressure data (if available)

• Standard documents

There are no relevant international standards.

2. METHOD

A. INTRODUCTION

This assay may be used to measure gene mutation in yeast, a eukaryotic micro-organism. Strains of *Saccharomyces cerevisiae* have been developed which detect forward or reverse mutations (base substitution and frameshift).

• Definitions

Base substitution mutagens are agents which cause a base change in the DNA. In a reverse mutation assay this change may occur at the site of the original mutation, or at a second site in the genome.

Frameshift mutagens are agents which cause the addition or deletion of single or multiple base pairs in the DNA molecule.

• Principle of the method

A variety of haploid and diploid strains of the yeast *Saccharomyces cerevisiae* can be used to measure the production of gene mutations induced by chemical agents.

Forward mutation systems in haploid strains such as the measurement of mutation from red, adenine-requiring mutants (ade-1, ade-2) to double adenine-requiring white mutants, and selective systems, such as the induction of resistance to canavanine and cycloheximide, have been utilized.

The most extensively validated reverse mutation system involves the use of the haploid strain XV 185-14C which carries the ochre nonsense mutations ade 2-1, arg 4-17, lys 1-1 and trp 5-48, that are reversible by base substitution mutagens inducing site-specific mutations or ochre suppressor mutations. XV 185-14C also carries the his 1-7 marker, a missense mutation reverted mainly by second site mutations, and the marker hom 3-10 which is reverted by frameshift mutagens.

The only extensively used diploid strain is D_7, which is homozygous for ilv 1-92.

B. DESCRIPTION OF THE TEST METHOD

- Preparations

Test substance

Solutions of the test substance and positive control substances should be prepared just prior to testing, using, when necessary, an appropriate vehicle. The final concentration of the vehicle should not significantly affect cell viability and growth characteristics.

Test strains

The haploid strain XV 185-14C and the diploid strain D_7 are the most widely used in gene mutation studies. Other strains may also be appropriate.

Media

Appropriate culture media are used for the determination of cell survival and mutant numbers.

Metabolic activation

Cells should be exposed to the test substance both in the presence and absence of an appropriate exogenous mammalian metabolic activation system.

The most commonly used system is a cofactor supplemented post-mitochondrial fraction from the livers of rodents pretreated with enzyme-inducing agents. The use of other species, tissues, postmitochondrial fractions, or procedures may also be appropriate.

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- Test conditions

Exposure concentrations

At least 5 adequately spaced concentrations of the test substance should be used. Among the factors to be taken into consideration are cytotoxicity and solubility. The lowest concentration must have no effect on cell viability. For toxic substances the highest concentration tested should not reduce survival below 5-10 per cent. Poorly water-soluble substances should be tested up to their limits of solubility, using appropriate procedures. For freely water-soluble, non-toxic substances, the highest concentration should be determined on a case-by-case basis.

Spontaneous mutation frequencies

Sub-cultures with spontaneous mutation frequencies within the accepted normal range should be used.

Number of replicates

At least 3 replicate plates should be used per concentration for the assay of prototrophs produced by gene mutation and for viability. In the case of experiments using markers such as hom 3-10 with a low mutation rate, the number of plates used must be increased to provide statistically relevant data.

Controls

Positive controls, using both a direct acting compound and a compound requiring metabolic activation, should be included in each study. A vehicle control should be used. The following are examples of substances which might be used as positive controls:

– methylmethanesulphonate, ethylmethanesulphonate, 4-nitroquinoline-N-oxide (direct acting compounds);
– N-nitrosodimethylamine, cyclophosphamide (indirect acting compounds);
– ICR-170 (direct acting frameshift mutagen).

- Performance of the test

Treatment of *Saccharomyces cerevisiae* is usually performed in a liquid test procedure using either stationary or growing cells. Initial experiments should be done on growing cells. $1 – 5 \times 10^7$ cells/ml are exposed to the test substance for up to 18 hours at 28°-37°C with shaking.

For experiments with metabolic activation, an adequate amount of metabolic activation system is added during treatment. At the end of the treatment, cells are centrifuged, washed and seeded upon appropriate culture medium. After incubation for 4-7 days at 28°-30°C in the dark, plates are scored for survival and the induction of gene mutation.

If the first experiment is negative, then a second experiment should be carried out using stationary phase cells. If the first experiment is positive, it is confirmed in an appropriate independent experiment.

3. DATA AND REPORTING

- Treatment of results

Data should be presented in tabular form, indicating number of colonies counted, number of mutants, survival and mutant frequency.

The data should be evaluated using appropriate statistical methods.

- Evaluation of results

There are several criteria for determining a positive result, one of which is a statistically significant dose-related increase in the number of mutants as well as in the mutant frequency.

Another criterion may be based upon the detection of a reproducible and statistically significant positive response for at least one of the test substance concentrations.

A test substance producing neither a statistically significant dose-related increase in the mutant frequency, nor a statistically significant and reproducible positive response at any one of the test substance concentrates is considered non-mutagenic in this system.

Both biological and statistical significance should be considered together in this evaluation.

- Test report

The test report should include the following information:

– strain used;

– test conditions: stationary phase or growing cells, composition of the media, incubation temperature and duration, metabolic activation systems;

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– treatment conditions: exposure levels, procedure and duration of treatment, treatment temperature, positive and negative control;

– number of colonies counted, number of mutants, survival and mutant frequency, dose-response relationship (if applicable), statistical evaluation of data;

– discussion of results;

– interpretation of results.

4. LITERATURE

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