

ICS 07.100
CCS C 53



**NATIONAL STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

GB 15193.12-2014

Replacing GB 15193.12-2003

**National Food Safety Standard -- HGPRT gene mutation test
(English Translation)**

Issued on: December 24, 2014

Implemented on: May 1, 2015

**Issued by: National Health and Family Planning Commission of the
People's Republic of China**

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Foreword

This standard replaces GB 15193.12-2003 "in vitro mammalian cell (V79/HGPRT) gene mutation assay." this standard compared with GB 15193.12-2003, the main changes are as follows.

- Standard name was changed to "national food safety standard in vitro mammalian cell gene mutation test HGPRT";
- Increasing the terms and definitions;
- Modified test purposes and principles;

- Increasing the method of preparation of different types of test substance;
- Increasing the reference positive control;
- Increased test cell lines.

National Food Safety Standard in vitro mammalian cell gene mutation test HGPRT

1 Scope

This standard specifies the in vitro mammalian cell hypoxanthine guanine phosphoribosyl transferase (HGPRT) gene mutation test basic test Test methods and technical requirements.

This standard applies to the evaluation of the test substance mutagenic effect.

2.1 HGPRT gene

Hypoxanthine guanine phosphoribosyl transferase gene in mammals. In humans, HGPRT gene located on the X chromosome length Arm coordinates Xq26.1; in mice also located in the X chromosome.

2.2 Mutation frequency

In some cell lines, a particular gene mutant cells (colonies) accounted cells (colonies) of the total percentage.

3 Test purposes and principles

Cells under normal culture conditions, capable of HGPRT-generating, selectively containing 6-thioguanine (6-thioguanine, 6-TG) of Culture medium, HGPRT catalyzes the production of nucleoside-5'-monophosphate (NMP), NMP incorporated into the DNA resulting in cell death. Carcinogenic and (or) Mutagen effect, the control structure of HGPRT gene mutation on the X chromosome in some cells can no longer produce HGPRT, thereby So that the mutant cells to 6-TG resistant effect, can survive in a selective growth medium containing 6-TG in.

In addition and without the addition of metabolic activation system conditions, the cells were exposed to test a certain time, then the cells were subcultured, Selective medium containing 6-TG, the mutant cells can continue to divide and form colonies. Based on the number of mutant colonies calculated mutation frequency to evaluate the mutagenicity of the test substance.

4.1 Cell

Common Chinese hamster lung cell line (V79) and Chinese hamster ovary cell line (CHO), others such as mouse lymphoma cells (L5178Y) and human lymphoblastoid cell lines (TK6) also. Cells should be carried out before use to check whether the mycoplasma contamination.

4.2 Broth

According to the test system should be used to select the appropriate cell type and medium. For V79 and CHO cells, common minimum essential medium Yang group (MEM, Eagle), Modified Eagle Medium (DMEM) with 10% fetal calf serum and an appropriate amount of antibiotics. For TK6 and L5178Y cells, commonly used RPMI1640 medium, 10% horse serum (culture flasks) or 20% horse serum (96 well plates) and an appropriate amount of antibiotics (penicillin, streptomycin).

4.3 Trypsin/EDTA solution

With calcium and magnesium in PBS, concentration of trypsin was 0.05%, the concentration of EDTA is 0.02% trypsin EDTA solution by Mixed 1. -20 °C storage.

4.4 Activation system

S9 mixture is generally used. S9 is prepared as follows.

Choose healthy male adult SD or Wistar rats weighing about 150g ~ 200g, weeks about five weeks to 6 weeks. The PCBs (Aroclor1254) was dissolved in corn oil at a concentration of 200g/L, according to 500mg/kg body weight by intraperitoneal injection once aseptically, 5d after death Animals were sacrificed before fasting 12h.

It may also be used phenobarbital and beta-naphthalene flavones and induction methods for preparing orally administered to rats fed sodium phenobarbital and beta-naphthalene Flavonoids, doses were 80mg/kg, continuous 3d, 16h after fasting animals were decapitated. Other operating with PCBs induction.

Animals were sacrificed after the liver was removed, weighed fresh ice-cold solution of potassium chloride (0.15mol/L) continuous flushing liver several times to remove it can inhibit the activity of microsomal hemoglobin. Per gram of liver (wet weight) was added potassium chloride solution (0.1mol/L) 3mL, along with an ice bath and transferred to a beaker the liver minced with sterile scissors in glass homogenizer (less than 4000r/min, 1min ~ 2min) or tissue homogenizer (less than 20000r/min, 1min) is made in the liver homogenates. The above operation should pay attention to the local cold and sterile environment.

The liver homogenates prepared at low temperature (0 °C ~ 4 °C) high-speed centrifuge at

9000g centrifugation 10min, the supernatant was aspirated S9 fraction, Aliquot in sterile freezer tubes or ampoules, each ampoule about 2mL, frozen with liquid nitrogen or dry ice rear -80 °C cryopreservation.

After S9 fraction prepared by the sterility test, measuring protein content (Lowry method) per milliliter protein content of not more than 40mg is appropriate, and by Indirect carcinogens (mutagens) Qualified identify their biological activity after storage in cryogenic freeze or dry, a period not exceeding one year.

S9 is used at a concentration of 1% to 10% (final concentration).

4.5 Selection agent

6- thioguanine (6-TG), recommend using a final concentration of 5µg/mL ~ 15µg/mL, formulated with sodium bicarbonate solution (0.5%).

4.6 Pretreated broth (THMG/T HG)

To reduce the frequency of spontaneous mutation of cells, prior to testing, the first cell culture plus 24h in culture medium containing THMG, the spontaneous killing the mutant cells, then cells were seeded in THG (without methotrexate in THMG broth) in cultured cells 1d ~ 3d to recovery Normal growth cycle and morphology.

THMG final concentration of each substance contained in the following (in addition to the culture medium ingredients outside).

- Thymidine, 5×10^{-6} mol/L;
- Hypoxanthine, 5×10^{-5} mol/L;
- Methotrexate, 4×10^{-7} mol/L;
- Glycine, 1×10^{-4} mol/L.

5.1.1 Formulated test substance

Solid test substances should be dissolved or suspended in a suitable solvent and diluted to the appropriate concentration. Liquid test substances can be used directly or diluted to Appropriate concentration. Test substance shall be obtained before using now, otherwise you must confirm the storage does not affect its stability.

5.1.2 Vehicle selection

Solvent must be non-mutagenic not react chemically with the test substance, does not affect cell survival and S9 activity. The preferred solvent is distilled Water; for water-insoluble test substance optionally other solvents, preferred dimethyl sulfoxide (DMSO), but it should not be used at a concentration greater than 0.5%.