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

GB 15193.20-2014

Replacing GB 15193.20-2003

National Food Safety Standard -- TK gene mutation test

食品安全国家标准 体外哺乳类细胞TK基因突变试验

(English Translation)

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Foreword

This standard replaces GB 15193.20-2003 "TK gene mutation test." this standard compared with GB 15193.20-2003, the main changes are as follows.

--- Standard name was changed to "national food safety standard in vitro mammalian cell gene mutation assay TK";

--- Modify the scope;

--- Increasing the terms and definitions;

- Modified test purposes and principles;
- Increasing the metabolic activation system;
- Increased THMG and THG selective media method of preparation;
- Increased CHAT CHT and selective media method of preparation;
- Increased trifluorothymidine method of preparation;
- Increased phosphate buffer preparation methods;
- Modify the test substance dose setting requirements;
- Modify control settings;
- Increase alternative cell lines and the corresponding test methods and data processing;
- Increasing the requirements of the test report;
- Expanded to explain the requirements of the test.

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

1 Scope

This standard specifies the in vitro mammalian thymidine kinase (thymidinekinase, TK) gene mutation test basic test methods and techniques Claim.

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

2.1 TK gene

Mammalian thymidine kinase gene. TK human genes located in the distal end of the long arm of chromosome 17; mice are located in the on the 11th chromosome.

2.2 Mutation frequency

In some cell lines, a particular gene mutant cells (colonies) occupy cells (colonies) of the total proportion (unit usually 10^{-6}).

3 Test purposes and principles

TK gene mutation test endpoint detection is TK gene mutations. TK gene mutation autosomal gene mutations.

TK thymidine kinase gene products in vivo catalytic generate thymidylate (TMP) reaction from thymidine (TdR). In normal circumstances Under this reaction it is not necessary for life, because the body mainly from the TMP-deoxy-uridine (dUMP), that is, from thymidylate dUMP methylation reaction synthesizing enzyme-catalyzed generation TMP. However, if the

thymidine analogue was added in cell culture (e.g. trifluorothymidine, i.e.

trifluorothymidine, TFT), the TFT can generate trifluorothymidine acid catalyzed thymidine kinase, and then incorporated into the DNA, causing death Mutation, so the cells can not survive. If the TK gene mutations that cause defects thymidine kinase, phosphorylation of the TFT can not, nor can be incorporated DNA, so the mutant cells in a culture medium containing the TFT can grow, that exhibit resistance to TFT. According to the number of colony forming mutations set, Calculate the mutation frequency and infer mutagenicity test substance. In the TK gene mutation test results can be found in two types of observation significantly different Colonies, namely large/small colonies (L5178Y cells) or normal growth/slow-growing colonies (TK6 cells), studies have shown that large colonies/Normal Growth colonies mainly by a point mutation or deletion of a small range of other causes, and small colony/colonies mainly by the slow growth of a wide range of abnormal chromosomes Change, or deletion of the gene involved in the regulation of cell proliferation caused.

4.1 Instrument

Commonly used laboratory equipment, a deep freezer (-80 °C) or liquid nitrogen tank, biological safety cabinet, incubator, inverted microscope, centrifuge.

4.2.1 Complete medium

RPMI1640 medium, 10% horse serum (culture flasks) or 20% horse serum (96 well plates) and the amount of antibiotic (Green Final concentration of neomycin, streptomycin were 100IU/mL and 100µg/mL).

4.2.2 THMG and THG selection medium

THMG medium. 3µg/mL thymidine (thymidine, T) 5µg/mL hypoxanthine (hypoxanthine, H) 0.1µg/mL methotrexate (methotrexate, M) 7.5µg/mL glycine (glycine, G).

THG medium. 3µg/mL thymidine (T) 5µg/mL hypoxanthine (H) 7.5µg/mL glycine (G).

Above a concentration of each reagent at a final concentration in the medium. Practical tests, often according to the method of Table 1 and THG dubbed THMG 100-fold concentration.

Configuration Table 1 T, H, M, G reagent Reagent relative molecular mass mass/mg and the volume of solvent concentration/(mg/mL) T 242.2 30 10mLH₂O 3 H 136.1 5 1mL1mol/LHCl 5 M 454.5 5 50mLH₂O 0.1 G 75.07 75 10mLH₂O 7.5 the above-described four kinds of reagents formulated in a 1000-fold concentration, this concentration then were taken of T, H, M, G 5mL each solution (20 mL total), or T, H, G solution each 5mL (a total of 15mL), were diluted with distilled water to 50mL, dubbed 100X THMG or THG stock solution.

Finally filtered through a membrane filter sterilization, packaging, stored at -20 °C. 1% of the proportion of complete medium when applying.

4.2.3 CHAT selection medium and CHT

CHAT media. 1×10^{-5} mol/L deoxycytidine (cytosinedeoxyriboside, C) 2×10^{-4} mol/L hypoxanthine (Hypoxanthine, H) 1×10^{-7} mol/L aminopterin (aminopterin, A) 1.75×10^{-5} mol/L thymidine (thymidine, T).

CHT medium. 1×10^{-5} mol/L deoxycytidine (C) 2×10^{-4} mol/L hypoxanthine (H) 1.75×10^{-5} mol/L Chest Glycosides (T).

Above a concentration of each reagent at a final concentration in the medium. Practical tests, often according to the method of Table 2 and CHT dubbed the CHAT 100-fold concentration.

Table Configuration 2 C, H, A, T reagent

Reagent relative molecular mass mass/mg and the volume of solvent concentration/(mol/L)
 C 227.2 113.6 50mLH₂O 1×10^{-2} H 136.1 1361.0 50mL1mol/LHCl 2×10^{-1} A 440.4 2.2
 50mLH₂O 1×10^{-4} T 242.2 423.85 10mLH₂O 1.75×10^{-2} the four kinds of reagents
 formulated in a 1000-fold concentration, and then were taken of the concentration C, H, A,
 T 5mL each solution (20 mL total), or C, H, T Solution of each 5mL (a total of 15mL), were
 diluted with distilled water to 50mL, dubbed 100X CHAT or CHT stock solution. Finally filter
 Membrane filtration sterilization, packaging, stored at -20 °C. 1% of the proportion of
 complete medium when applying.

4.3.1.1 Magnesium potassium solution

1.9g of magnesium chloride and potassium chloride 6.15g add distilled water to 100mL.

4.3.1.2 0.2mol/L phosphate buffer (pH 7.4) Disodium hydrogen phosphate
 (Na₂HPO₄, 28.4g/L) 440mL, sodium dihydrogen phosphate (NaH₂PO₄ · H₂O, 27.6g/L)
 60mL, pH adjusted 7.4, 0.103MPa 20min sterilization or to filter bacteria.

4.3.1.3 Coenzyme -II (oxidized) solution

Under sterile conditions weigh coenzyme -II, with sterile distilled water formulated to
 0.025mol/L solution. Using now.

4.3.1.4 Glucose-6-phosphate sodium salt solution

Glucose-6-phosphate sodium salt was weighed, dissolved in distilled water formulated as
 0.05mol/L, sterilized by filtration. Using now.