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

GB 15193.10-2014

Replacing GB 15193.10-2003

**National Food Safety Standard -- Unscheduled DNA synthesis
test**

(English Translation)

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Foreword

This standard replaces GB 15193.10-2003 "unscheduled DNA synthesis test." this standard compared with GB 15193.10-2003, the main changes are as follows.

- Standard name was changed to "national food safety standard in vitro mammalian cell DNA damage repair (unscheduled DNA synthesis) test";
- Modified test purposes and principles;
- Revised test methods;
- Modify the data processing;
- Modify the results of the evaluation.

National Food Safety Standard in vitro mammalian cell DNA damage repair (Unscheduled

DNA synthesis) test

1 Scope

This standard specifies the in vitro mammalian cell DNA damage repair (unscheduled DNA synthesis) test basic test methods and techniques Claim.

This standard applies to the evaluation of the test substance mutagenicity and (or) carcinogenic.

2.1 Unscheduled DNA synthesis

When the damaged DNA, DNA repair synthesis mainly in other cell cycle other than S phase, said the unscheduled DNA synthesis.

3 Test purposes and principles

Under normal circumstances, DNA synthesis only in the mitotic cycle S on schedule. When chemical or physical agent-induced DNA damage After the cells start unscheduled DNA synthesis procedures to repair DNA damage in the area. Primary mammalian non S Isolation, Culture Cells or continuous cell lines added 3H- thymidine (3H-TDR), autoradiography or liquid scintillation counting technique by DNA radiation (LSC) was detected in cells exposed to the amount of 3H-TDR incorporation of DNA, and may indicate the extent of damaged DNA repair synthesis.

In cultured cells, with the lack of semi-essential amino acid arginine medium (ADM) synchronize culture, DNA synthesis initiating Blocked, the cells were synchronized in G1 phase; and inhibit residual semi-conservative DNA replication with drugs (commonly used hydroxyurea), the incorporation by 3H-TDR it can display unscheduled DNA synthesis (UDS).

4.1 Reagents

Note: Unless otherwise indicated, all the reagents, were of analytical grade, test water is double distilled water or ultrapure water.

4.1.1 Cell growth medium

Medium Eagle's minimum requirements (Eagle'sMinimalEssentialMedium, EMEM), 10% fetal calf serum, penicillin Su, streptomycin stock solution, final concentration of penicillin is 100 units, streptomycin at a final concentration of 100µg/mL. EMEM medium You can choose a variety of powder medium supply of goods by manufacturers to provide information on the preparation and sterilization. 4 °C refrigerator storage.

4.1.2 Synchronization cell culture medium

The arginine-free EMEM medium (the ADM), was added calf serum, penicillin, streptomycin with cell density growth medium.

4.1.3 Hanks balanced salt solution (the HBSS)

4.1.3.1

accumulator A. Sodium chloride 160g, potassium chloride 8g, magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) 2g and magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) 2g dissolved in 800mL distilled water (50 °C ~ 60 °C) in. Another 2.8g anhydrous calcium chloride dissolved in 100mL double-distilled water. The above two After the mixed solution, add water to 1000mL, chloroform was added 2mL, stored at 4 °C refrigerator.

4.1.3.2

accumulator B. The disodium hydrogenphosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) 3.04g (or $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ 1.2g), potassium dihydrogen phosphate ($\text{KH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$) 1.2g (or KH_2PO_4 0.95g), 20g of glucose were dissolved in double distilled water 800mL, 100mL added 0.4% phenol red solution [phenol red take 1g, dissolved in 3mL sodium hydroxide (1mol/L) in], until completely dissolved, double distilled water to 250mL, add water to 1000mL, chloroform was added 2mL, stored at 4 °C refrigerator.

4.1.3.3

reservoir C. 1.4% sodium bicarbonate in distilled water preparation.

Preparation 4.1.3.4 Application Solution. Take 1 part liquid A, B solution 1, 18 parts of water, mixed packed in glass containers; autoclaving, 4 °C ice Save me. Before using the solution C was added to adjust the pH to 7.2 to 7.4.

4.1.4 Without calcium, Dulbecco's phosphate buffer magnesium

pH7.4, take the sodium chloride 8.00g, potassium dihydrogen phosphate (KH_2PO_4) 0.20g, KCl 0.20g, disodium hydrogen phosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) 2.89g dissolved and dilute to 1000mL double distilled water.

4.1.5 0.02% ethylene diamine tetraacetate tetrasodium or disodium (EDTA) solution of Take EDTA 0.2g dissolved calcium, magnesium phosphate buffer, the volume to 1000mL. Autoclaving after use.

4.1.6 Antibiotic stock solution

Take clinical injection of penicillin G100 million units and streptomycin 1g powder under aseptic dissolved in 100mL of sterile distilled water to a concentration of 1 million units of penicillin, streptomycin concentration for 10000μg/mL, 100mL each added to the medium

when using antibacterial Prime stock solution 1mL.

Preparation of components 4.1.7 rat liver microsomes-9 S S-9 mixture may be formulated in the following manner. The disodium hydrogenphosphate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) 86.8mg, monopotassium phosphate 7.0mg, Magnesium chloride ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) 8.1mg, 6- glucose phosphate (G-6-P) 5.4mg, coenzyme II (CoII) (purity 90%) 4mg dissolved ADM was added N-2- hydroxyethyl piperazine--N-2- ethanesulfonic acid (HEPES) solution (1mol/L) 0.2mL rat liver S-9 and components 0.8mL ~ 4mL or 20mL, and adjust the pH with sodium bicarbonate solution to 7.2 to 7.4.

4.1.8.1 KodakD-170 developer

Reservoir. Anhydrous sodium sulfite 25g, Potassium bromide 1g, water was added to 200mL. When diluted with water use, into 2-amino-phenol hydrochloride (Amitol) 4.5g, dilute to 1000mL.

4.1.8.2 KodakD-196 developer

Water (50 °C) 500mL, dissolved sequentially metol (sulfuric acid methyl amino phenol) 2g, anhydrous sodium sulfite 72g, hydroquinone 8.8g, Anhydrous sodium carbonate 48g, potassium bromide 4g, dilute to 1000mL.

4.1.8.3 Stop Bath

98% glacial acetic acid 15mL, dilute to 1000mL.

4.1.8.4 KodakF-5 Fixer

Water (50 °C) 100mL, successively dissolved Hyperion 240g, anhydrous sodium sulfite 15g, 28% acetic acid 48mL, boric acid 7.5g, potassium alum 15g, dilute to 1000mL.

4.1.9 Perchloric acid (0.25mol/L and 0.5mol/L)

70% perchloric acid 8.57mL, add water to 100mL, concentration of 1mol/L.

4.1.10 Scintillation fluid

Weigh 2,5-diphenyl oxazole (PPO) 5g, 1,4- bis - [5-phenyl-oxazol-2-yl] - benzene (POPOP) 300mg was dissolved in toluene, the volume to 1000mL.

4.2 Material

Recommend the use of disposable cell culture vessels before and microporous membrane, general test equipment should use cell culture techniques requires cleaning package Tie, disinfection and sterilization. On the glass with a rubber stopper and metal utensils available without high-temperature sterilization, the temperature rose to 140 °C, the holding 2h.