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

SN/T 2497.24-2010

**Test method of import and export dangerous chemicals. Part
24: Vitro test of cell immunologic function**

进出口危险化学品安全试验方法 第24部分：细胞免疫功能体外检测方法
(English Translation)

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Foreword

SN/T 2497 "Test Methods for Chemical Safety & Exports dangerous" series of standards is divided into 29 parts.

- Part 1. mammalian liver cells in vivo DNA synthesis (UDS) test;
- Part 2. plaque-forming cells (PFC) test;
- Part 3. Large Zhuan reproduction test;
- Part 4. *Saccharomyces cerevisiae* mitotic recombination test;
- Part 5. testicular cells UDS test;
- Part 6. mammalian cells in vitro sister chromatid exchange test;
- Part 7. mouse ear swelling test;
- Part --- Article 8. fossa lymph node assay;
- Part 9. Determination of serum hemolysin test;
- Part --- Article 10. T lymphocyte proliferation assay test;
- Part 11. germline mutation assay;
- Part 12. single cell gel electrophoresis test;
- Part 13. fluorescence in situ hybridization;
- Part --- Article 14. SDS- polyacrylamide gel electrophoresis;
- Part 15. PCR-SSCP experiment;
- Part --- Article 16. Western-Blot test;
- Part 17. Behavior of mammalian toxicology tests;

- Part 18. Detection of DNA adducts;
- Part 19. NorthernBlot experiment;
- Part 20. Bradford protein assay;
- Part 21. agarose gel electrophoresis;
- Part 22. T_m value determination method of DNA;
- Part 23. organelle separation experimental methods;
- Part 24. cellular immunity in vitro assay method;
- Part 25. humoral immune function tests;
- Part 26. macrophage function tests;
- Part 27. Flow cytometry of apoptosis;
- Part 28. Application shuttle plasmid mutations Inspection;
- Part 29. Biochemical Oxygen Demand (BOD) was measured.

This part is Part 24 SN/T 2497 series of standards.

The partial modification of the use of "Toxicology test methods and techniques" immune toxicology research method, technical content and the method described above Exactly the same, in a standard text format according to GB/T 1.1 made editorial changes.

This section proposed and managed by the National Certification and Accreditation Administration Committee.

This section is responsible for drafting unit. People's Republic of China Tianjin Entry-Exit-Entry Inspection and Hunan People's Republic of China Quarantine Bureau.

1 Scope

SN/T 2497 provisions of this part of the test principle and export of dangerous chemicals in the immune function test in vitro tests, test Test methods and test results.

This section applies to in vitro testing for immunotoxicity testing and export of dangerous chemicals in cells.

2 Test principle

Human peripheral blood T lymphocytes have receptors sheep erythrocytes (E receptor), that CD2 molecules, and B lymphocytes are not.

Thus in vitro, when mixed with sheep red blood cells, the T cells can be formed as the center, surrounded by sheep red blood cells Like garlands of roses. This test can be used to

detect the number and proportion of peripheral blood T lymphocytes.

Test Method 3

3.1 Reagents

3.1.1

lymphocyte separation medium, heparin, calcium, magnesium, Hank's solution, pH value of 7.2 ~ 7.6, Alsever solution, 0.8% glutaraldehyde, 0.2% cold Meilan.

3.1.2 Sheep red blood cells

Fresh sheep blood was washed with a solution to save Alsever, formulation of 1% sheep red blood cell suspension.

3.1.3 Absorption inactivated fetal bovine serum

After inactivated fetal bovine serum after 56 °C 30min per ml of fetal calf serum was added to the washed sheep red blood hematocrit 0.1mL, mixing, 37 °C for 30min, then 2000r/min centrifugal 20min, supernatant at 4 °C.

3.2 Test procedure

3.2.1

take anti-clotting heparin 3mL, plus 3mLHank's solution to fold diluted blood was added slowly to the solution of lymphocyte separation along the wall on, 2000r/min centrifugal 20min.

3.2.2

Carefully draw isolated white liquid interface layer of mononuclear cells (containing lymphocytes), washed with Hank's solution twice, to adjust the cell at a concentration of 2×10^6 cells/mL spare.

3.2.3

lymphocyte suspension was added 0.1mL Fahrenheit in a new tube, the absorption inactivated fetal bovine serum 0.1mL, 1% sheep red blood cells Suspension 0.1mL mix, 37 °C incubated for 5min, then 500r/min centrifugal 5min. The tubes were placed in 4 °C refrigerator, at least 2h.

3.2.4

Remove the test tube, the supernatant was discarded part, gently rotate the tube Fahrenheit cells mix, then along the wall of one drop of 0.8% glutaraldehyde, Placed 4 °C refrigerator 20min.

3.2.5

gently draw sheet 1 drops, 1 drop of 0.2% plus cold methylene blue dye 5min, cover glass, high magnification observation rosette formation Happening.

4 Results

Where the adsorption lymphocytes around 3 or more persons shall E- SRBC rosette forming positive lymphocyte count of 200, Percentages E- rosette formation, see equation (1).