

ICS 13.300
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**INDUSTRY STANDARD OF THE
PEOPLE'S REPUBLIC OF CHINA**

SN/T 2497.23-2010

**Test method of import and export dangerous chemicals. Part
23: Separation of cellular organelles**

进出口危险化学品安全试验方法 第23部分：细胞器的分离实验方法
(English Translation)

Issued on: March 2, 2010

Implemented on: September 16, 2010

Issued by: General Administration of Customs

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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 is Part 23 SN/T 2497 series of standards.

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 separation of experimental methods import and export of dangerous chemicals organelles test, the test sample, the test Test solutions and test procedures.

This section applies to the import and export of dangerous chemicals isolated experimentally test organelles.

2 Terms and definitions

The following terms and definitions apply to this section.

Bilayer membrane organelles of eukaryotic cells, intracellular oxidative phosphorylation and ATP formed the main place.

When cells were homogenized crushed, after rupture of the membrane structure endomembrane system themselves up reclosable vesicles (mainly in the endoplasmic reticulum and Golgi Body), these vesicle diameter of about 100nm or so, are heterogeneous aggregates.

In the cell membrane surrounded by a tunnel system, it is an important cell organelles.

Principle 3

The proportion of cells of different sizes and structures are not the same, the centrifugal sedimentation rate is not the same, according to this principle, by differential centrifugation, First with a low speed larger particles precipitate, and then a high speed, the float in the supernatant of the precipitated particles, so that a variety of cell structures, such as the nucleus, mitochondria can be separated.

4.2 RSB solution

Tris · Cl final concentration of 10mM; MgCl₂ final concentration of 3mM; KCl to a final concentration of 10mM. The pH was adjusted with dilute hydrochloric acid to 7.4, after autoclaving, 4 °C preservation.

The content of each component of the solution. D- sorbitol final concentration of 2M; HEPES at a final concentration of 10mM; EDTA to a final concentration 2mM. Value with dilute NaOH solution to adjust the pH to 7.4, membrane filtration sterilization, 4 °C preservation.

5.1 Cell lysis

Cells were resuspended RSB solution, 1.10 volume ratio of 3% NP-40, repeatedly mixing, the ice bath was 10min.

5.2 Separation of nucleus and mitochondria

In the ultracentrifuge, the cell lysate was 5000r/min centrifugal 20min, the supernatant was collected;