High Efficiency Nuclei Extraction Kit User Manual

1 Product Information

Product Name	Model	Specification
High Efficiency Nuclei Extraction Kit	DHNE-10	10T
	DHNE-2511	25T

2 Description

Preparation of extracts from the nucleus is often the first step in many cell biology studies, High Efficiency Nuclei Extraction Kit (High purity) is designed for the extraction of pure and fragile nuclei from mammalian cells and tissues, which can quickly isolate nuclei from mammalian cultured cells and homogenate tissues for subsequent experimental studies.

Main principle: Fresh or frozen tissue samples are dissociated into mononuclear suspension by a combination of mechanical dissociation and tissue lysis of the extracellular matrix. The single cell suspension dissociator (RWD) is chiefly used for mechanical dissociation, while the High Efficiency Nuclei Extraction Kit mainlydigests the tissue primarily by lysis. The mononuclear suspension obtained was mainly used in cell apoptosis, proteomics analysis, mononuclear gene expression analysis and nuclear sequencing.

3 Components

4 vials of reagents in total, including:

1 bottle of Buffer A
1 bottle of Buffer B
1 bottle of Buffer C
1 bottle of Buffer D

4 Test Capacity

Product Model / Weight of tissue	DHNE-10	DHNE-2511
0.05 ~ 0.2 g (included)	20 T	50 T
0.2 ~ 1 g	10 T	25 T

5 Transport and Storage

Transport at -25 ~ -15℃. Store one component (Buffer D) in the kit at -25 ~ -15°C, and the other three components (Buffer A, Buffer B and Buffer C) at 2 ~ 8°C, with a validity period of 12 months (avoid repeated freezing and thawing and vibrate agitation).

6 Requirements for Reagents and Instruments

Ice machine
40 μm cell strainer
70 μm cell strainer
Tissue processing tube* (RWD)
Single Cell Suspension Dissociator (RWD)

7 Method for Use

7.1 Tissue Lysis Mixture Preparation

Prepare the Tissue Lysis Mixture in the Tissue processing tube according to the following table (various reagents need to be pre-cooled on ice), and the mixture is freshly prepared just before use. The mixture can treat 0.05 ~ 1.0 g common tissues and tumor tissues of mice. When working with more than the above tissue, the number of tissue processing tubes needs to be increased.

Weight of tissue	Tissue lysis mixture
0.05 ~ 0.2 g (included)	1.8 mL Buffer A + 0.2 mL Buffer D
0.2 ~ 1 g	3.6 mL Buffer A + 0.4 mL Buffer D

7.2 Protocol for Tissue Lysis (always on ice)

(1) Add corresponding mixture in the tissue processing tube according to the above tissue lysis mixture preparation table.

(2) Cut the tissue into small pieces of 2 ~ 4 mm size, store the tissue with pre-cooled PBS and weight the tissue with an electronic balance for tissue of the target weight.

⚠ Note: Observe the texture of the sample during cutting, and the fat tissue and connective tissue of the sample can be cut as much as possible.

(3) Transfer the tissue into the tissue processing tube with the mixture.

(4) Tighten the tissue processing tube, invert it and mount it into the bushing of the single cell suspension dissociator.

⚠ Note: make sure the sample is located in the rotor/stator area.

(5) Run the program **Nuclei-1-N**.

(6) After the program is completed, remove the tissue processing tube from the single cell suspension dissociator and locate it on ice for lysis for 10 min.

(7) Wet a 70 μm cell strainer with 1 mL of pre-cooled PBS, and filter the cell suspension sample with the wetted cell strainer, and collect the cell suspension in a 50 mL centrifuge tube.

(8) Rinse the tissue processing tube with 3 mL PBS, after filtering through a 70 μm filter, collect it in the 50 mL centrifuge tube in step (7).

(9) Centrifuge the cell suspension at 500×g for 5 minutes and completely discard the supernatant.

(10) Resuspend the cells with 1 mL Buffer B (If there is more precipitation, the volume of Buffer B can be increased).

(11) Filter the re-suspend cell precipitate with a wetted 40 μm cell strainer, and collect the cell suspension in another centrifuge tube.

(12) Centrifuge the cell suspension at 500×g for 5 minutes and completely discard the supernatant.

(13) Resuspend the cell precipitate with 1 mL Buffer C for the mononuclear suspension, which was immediately used in the next experiment.

⚠ Note: For clearer brain tissues and lung tissues, add 3 mL 1M sucrose at the bottom of a 15 mL tube, then slowly add 1 mL suspension on the upper layer of the sucrose, and centrifuge it in 4℃, 3000×g, accel 9 decel 3 for 15 min. completely discard the supernatant and the cell precipitate with 1 mL Buffer C for the mononuclear suspension.

(Optional) If temporary storage is required, add Rnase inhibitor (concentration 1 U/μL) in the mononuclear suspension and stored in the refrigerator at -80℃.

8 Precautions

(1) This kit is valid for 12 months, and RWD shall not guarantee the validity of expired products.

(2) If the tissue lysis should be operated in 4℃, if a downstream experiment is required, add Rnase inhibitor (concentration of 1 U/μL) to the resuspension and transfer the nuclei to -80°C or liquid nitrogen for storage;

*Note: The tissue processing tubes of RWD are not available in the USA market.

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