High Efficiency Nuclei Extraction Kit Instructions

Product Information

Product Name	Model	Specification
High Efficiency Nuclei Extraction Kit	DHNE-2511	25 T
High Efficiency Nuclei Extraction Kit (Trial Pack)	DHNE-10	10 T

Description

High Efficiency Nuclei Extraction Kit (the "Kit") can prepare fresh or frozen cells and tissue of mammals into single-cell nucleus suspensions quickly and efficiently. This optimized protocol can help obtain as many single-cell nucleus suspensions with intact nuclei as possible. The single-cell nucleus suspensions can be used to analyze apoptosis, proteomics, mononuclear gene expression and nuclear sequencing.

Main principle: Mechanical dissociation is in combination with tissue lysis to prepare fresh or frozen cells and tissue of mammal into single-cell nucleus suspensions quickly and efficiently. RWD Single Cell Suspension Dissociator mainly plays a role in mechanical dissociation, while the Kit mainly digests the tissue through lysis.

Components

Product Name	Components	Specification	Storage Condition
High Efficiency Nuclei Extraction Kit	Buffer A (solution)	1 vial	2℃ ~ 8℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃
	Buffer C (solution)	1 vial	2℃ ~ 8℃
	Buffer D (solution)	1 vial	-25℃ ~ -15℃
High Efficiency Nuclei Extraction Kit (Trial Pack)	Buffer A (solution)	1 vial	2℃ ~ 8℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃
	Buffer C (solution)	1 vial	2℃ ~ 8℃
	Buffer D (solution)	1 vial	-25℃ ~ -15℃

Test Capacity

✓ Recommendation for single process of tissue:

Tissue Type	Capacity	Capacity-Trial Pack	Initial Sample Dosage
Cells / Tissue of Mammals	50 T	20 T	($1*10^6$ ~ 10^7) / 0.05 ~ 0.2 g (include)
	25 T	10 T	(> 10^8) / 0.2 ~ 1.0 g

Storage & Transportation

✧ Transported at -25℃ ~ -15℃.

✧ The Kit is separated into two packages due to different storage temperature, please store them separately according to the attached temperature label.

✧ It is recommended that the buffer D should be dissolved separately, mix evenly and stored in small packages. Avoid repeated freezing, thawing and shaking.

✧ The Kit is valid for 12 months from the date of manufacture.

Reagent & Instrument

Reagent	PBS Buffer	RPMI 1640 or DMEM Medium	DAPI Stain
Consumable	40 μm Cell Strainer	70 μm Cell Strainer	Tissue Processing Tube (RWD)
	0.22 μm Syringe Filter (optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	High Speed Refrigerated Centrifuge (RWD: #M1416R)	

Operation

Preparation

Preparation of tissue lysis mixture: 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 should be freshly prepared just before use. The mixture can be used to treat 0.05 ~ 1.0 g common tissue of mice, tumor tissue and cells of mammals. When dealing with tissue heavier than the above weight, the number of tissue processing tubes need to be increased; when dealing with cells of mammals, the mixture should be blended well before adding the cells.

Tissue / Cells Weight	Mixture
($1*10^6$ ~ 10^7) / 0.05 ~ 0.2 g (include)	1.8 mL Buffer A + 0.2 mL Buffer D
(> 10^8) / 0.2 ~ 1.0 g	3.6 mL Buffer A + 0.4 mL Buffer D

Mechanized Protocol (On ice)

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

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

⚠ Note: Observe the texture of the sample and the fat tissue and connective tissue of the sample is suggested to be cut off as much as possible.

(3) Transfer the tissue to the tissue processing tube containing the mixture.

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

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

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

⚠ Note: Cell lysis starts from this step.

(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 (or place the tube in the 4℃ refrigerator for 10 min).

(7) Wet the 70 μm cell strainer with 1 mL of pre-cooled buffer B, filter the cell suspension through the cell strainer and collect the cell suspension to the 50 mL centrifuge tube.

(8) Rinse the tissue processing tube with 3 mL buffer B, filter the suspension through the 70 μm cell strainer and collect it to the 50 mL centrifuge tube mentioned in step (7).

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

(10) Resuspend the cell suspension with 1 mL buffer B (If there is much precipitate, the volume of buffer B can be increased).

(11) Filter the resuspended cell suspension through the 40 μm cell strainer, and collect the cell suspension to another centrifuge tube.

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

(13) Resuspend the cell suspension with 1 mL buffer C for gaining the mononuclear suspension, which is immediately used in the next experiment.

⚠ Note: For clearer brain tissue and lung tissue, add 3 mL 1M sucrose at the bottom of a 15 mL tube, then slowly add 1 mL suspension on the surface of the sucrose, and centrifuge the tube in 4℃, 3000×g, acceleration of 9 and deceleration of 3 for 15 min. After centrifugation, completely discard the supernatant and resuspend the suspension with 1 mL buffer C to gain 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℃.

Manual Protocol

(1) Add corresponding mixture in the tissue processing tube according to the above table in ***"Preparation"***.

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

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

(3) Transfer the tissue to the petri dish or the 50 mL centrifuge tube containing the mixture.

⚠ Note: Cell lysis starts from this step.

(4) Use a grinding rod or syringe to manually grind tissue for 2 ~ 5 min.

(5) After grinding, place the petri dish on ice for 30 min or in the refrigerator at 4℃ for 30 min. Use the pipette to blow the suspension 10 times every 10 min.

(6) Filter the cell suspension through the 70 μm cell strainer and collect the cell suspension to the 50 mL centrifuge tube.

(7) Rinse the cell strainer with 3 mL buffer B, centrifuge all the cell suspensions at 4℃, 500×g for 5 min and completely discard the supernatant.

(8) Resuspend the cell suspension with 2 mL buffer B, filter the cell suspension through the 40 μm cell strainer, and collect the cell suspension to the centrifuge tube. Turn the centrifuge tube up and down 10 times.

(9) Centrifuge each group of suspension at 4℃, 500×g for 5 min and completely discard the supernatant. Resuspend the cell suspension with 1 mL buffer C.

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

⚠ Note: Compard with mechanized protocol, the manual protocol has a certain fluctuation of cell number and incomplete digestion. It is suggested to adjust the time of digestion and blowing according to actual condition.

Precautions

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

(2) The tissue lysis should be operated in 4℃. If 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.

(3) If RNA needs to be extracted, please add RNase inhibitor (concentration of 1 U/μL) into buffer A and buffer C before extraction.

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

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