High Activity Neonatal Mouse and Rat Heart Enzymatic Digestion Kit-25T Instructions

Product Information

Product Name	Model	Specification
High Activity Neonatal Mouse and Rat Heart Enzymatic Digestion Kit-25T	DHNCE-2518	25 T

Product Description

High Activity Neonatal Mouse and Rat Heart Enzymatic Digestion Kit-25T (the "Kit") can prepare heart tissue of neonatal mice or rats (P0-P3) into single cell suspension gently, quickly, and efficiently. This optimized protocol can help obtain as many single cell samples with high cell viability as possible, while preserving the important surface epitopes of cells. The obtained single cell suspension can be applied in downstream experiments such as cell culture or sorting of cardiomyocyte, fibroblast and endothelial cell, flow cytometry analysis, and single cell sequencing.

Main principle: Mechanical dissociation is in combination with enzymatic digestion of the extracellular matrix (while maintaining the intactness of tissue structure) to prepare the neonatal heart tissue into single cell suspension. RWD Single Cell Suspension Dissociator is chiefly used for mechanical dissociation, while the Kit mainly digests the tissue through enzymatic digestion. After dissociation, the cell suspension is filtered through the cell strainers to remove tissue residues to obtain single cell suspension.

Product Components

Product Name	Components	Quantity	Storage
High Activity Neonatal Mouse and Rat Heart Enzymatic Digestion Kit-25T	Enzyme A Reagent (powder)	1 vial	2 ~ 8°C
	Enzyme B Reagent (powder)	1 vial	2 ~ 8°C
	Enzyme C Reagent (powder)	1 vial	-25 ~ -15°C
	Buffer C (solution)	1 vial	2 ~ 8°C
	Buffer D (solution)	2 vial	2 ~ 8°C

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Neonatal Mouse and Rat Heart Tissue	25 T	2 ~ 20 neonatal mice, or ≤ 10 neonatal rats to be processed per time

Storage & Transportation

- ✧ The Kit should be transported at 2 ~ 8°C.
- ✧ It is recommended that all the enzyme reagents should be dissolved separately until clear, mixed evenly and stored in small packages (Enzyme B needs to be dissolved in the 37°C water bath). Avoid repeated freezing, thawing and vigorous shaking.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagents & Instruments

Reagents	HBSS (with Ca^{2+} and Mg^{2+})	FBS
	DMEM	Red Blood Cell Lysis Buffer (Optional)
Consumables	Tissue Processing Tube (RWD)	Heater (RWD: #HJ-400)
	70 μm Cell Strainer	0.22 μm Syringe Filter (Optional)
Instruments	Single Cell Suspension Dissociator (RWD)	High-Speed Benchtop Refrigerated Centrifuge (RWD: #M1416R)

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve enzyme A reagent (powder) with 2.7 mL of HBSS (with $Ca^{2+}$and Mg^{2+}), subpackage the solution, and store at -25 ~ -15°C. The enzyme solution can be stored stably for 6 months at -25 ~ -15°C and it should avoid repeated freezing, thawing and vigorous shaking.

(2) Preparation of enzyme B solution: Dissolve enzyme B reagent (powder) with 2.7 mL of HBSS (with $Ca^{2+}$and Mg^{2+}) until clear in the 37°C water bath, subpackage the solution, and store at -25 ~ -15°C. The enzyme solution can be stored stably for 6 months at -25 ~ -15°C and it should avoid repeated freezing, thawing and vigorous shaking.

(3) Preparation of enzyme C solution: Dissolve enzyme C reagent (powder) with 1 mL of buffer C, subpackage the solution, and store at -25 ~ -15°C. The enzyme solution can be stored stably for 6 months at -25 ~ -15°C and it should avoid repeated freezing, thawing and vigorous shaking.

Mechanized Protocol

(1) Depending on the tissue type, prepare the enzyme mixture in the tissue processing tube. If cell culture is required, the enzyme mixture should be sterile-filtered through a 0.22 μm syringe filter. After filtration, the total volume of the mixture should be 2.5 mL. The subsequent operation should be completed on the aseptic operating platform. For detailed proportion of preparation, refer to Table 1:

Table 1 Preparation proportion of enzyme mixture

Buffer D	Enzyme A	Enzyme B	Enzyme C
2.275 mL	100 μL	100 μL	25 μL

⚠ Note: Enzyme B solution should be fully dissolved in the 37°C water bath until clear before preparing enzyme mixture.

(2) After obtaining the heart tissue sample, place the sample in a petri dish containing pre-cooled PBS or DMEM for short-term preservation. Gently remove the blood vessels or blood streaks and adipose tissue from the surface of the sample using an ophthalmic forceps.

⚠ Note: To obtain higher purity of cardiomyocytes, dissociate only the cardiac apex tissue (the cardiac apex is located at the ventricular end of the heart and constitutes approximately 1/3 of the heart's mass) and the heart tissue should be prepared just before use.

(3) Take an appropriate amount of tissue and transfer to the tissue processing tube containing enzyme mixture.

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

⚠ Note: Ensure that the sample is in the area where the rotor/stator is located.

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(5) Run the program **M_NeoHeart_Heater_1**.

(6) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator.

(7) Wet the 70 μm cell strainer with 1 mL of pre-cooled DMEM containing 10% FBS, filter the cell suspension through the wetted cell strainer, and collect it to a 50 mL centrifuge tube.

(8) Rinse the tissue processing tube with 10 mL of pre-cooled DMEM containing 10% FBS, filter the cell suspension through the 70 μm cell strainer, and collect it to the 50 mL centrifuge tube in step (7).

⚠ Note: For a small amount of tissue, centrifugation can be performed in a 15 mL centrifuge tube.

(9) Centrifuge the cell suspension at 600×g for 5 min under 4°C. After centrifugation, carefully discard the supernatant to avoid aspirating out the cell pellet.

(Optional) If the removal of red blood cells is required, resuspend the cells treated in step (9) with 1 mL of 1× red blood cell lysis buffer, then place on ice and incubate for 1 ~ 2 min. Then, resuspend with 10 mL of pre-cooled DMEM containing 10% FBS to terminate the lysis, centrifuge the cell suspension at 600×g for 5 min, and completely discard the supernatant.

⚠ Note: When the downstream experiment is the primary cell culture, the red blood cell lysis is not required.

(10) Resuspend the cells with DMEM or other buffers to required volume for subsequent experiments.

Manual Protocol

(1) Follow the steps (1) to (3) of ***Mechanized Protocol*** to process the tissue sample.

(2) Place the tissue processing tube containing tissue and enzyme into the 37°C water bath and incubate at 50 rpm for 10 min. After incubation, pipette to mix the cell suspension 10 times with a 1 mL pipette, which the tip is cut off approximately 0.5 cm.

⚠ Note: In manual protocol, the tissue processing tube can be replaced by the 50 mL centrifuge tube.

(3) Repeat step (2) two times.

(4) Follow the steps in ***Mechanized Protocol*** from steps (7) to (10).

⚠ Note: Compared with mechanized protocol, the manual protocol has a certain fluctuation of cell number and incomplete digestion. It is recommended to adjust the time of digestion and the times of pipetting according to the experimental conditions.

Precautions

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

(2) For any downstream cell culture to be performed subsequent to tissue dissociation, it is necessary to ensure that all steps are performed under sterile conditions.

(3) The enzyme reagent should be stored in small packages, and avoid repeated freezing and thawing. It should be used after dissolving on ice or in a refrigerator at 4°C to maintain its activity.

(4) Standard culture conditions for primary cardiomyocytes: DMEM/F-12 medium (containing 1X Glutamine) + 10% FBS + 1% P/S. To enhance cardiomyocyte purity, differential plating can be performed. After plating for 2 h, the supernatant is collected and centrifuged at 300×g for 5 min. The cell pellet is then resuspended in fresh culture medium and subjected to a second plating to obtain roughly purified cardiomyocytes.

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