High Activity Neonatal Brain Enzymatic Digestion Kit (Mouse & Rat) Instructions

Product Information

Product Name	Model	Specification
High Activity Neonatal Brain Enzymatic Digestion Kit (Mouse & Rat)	DHNBE-5002	50 T

Product Description

High Activity Neonatal Brain Enzymatic Digestion Kit (Mouse & Rat) (the "Kit") can prepare neonatal brain tissue of mouse and rat (fetal rat brain or neonatal brain P≤7) 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 maintaining the important surface epitopes of cells. The single cell suspension can be applied in downstream experiments such as cell sorting, primary cell culture 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 brain 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 strainer to remove tissue residues to obtain single cell suspension.

Components

Product Name	Components	Quantity	Storage Condition
High Activity Neonatal Brain Enzymatic Digestion Kit (Mouse & Rat)	Enzyme A Reagent (powder)	1 vial	2℃ ~ 8℃
	Enzyme B Reagent (powder)	1 vial	-25℃ ~ -15℃
	Buffer A (solution)	2 vial	2℃ ~ 8℃
	Buffer B (solution)	1 vial	2℃ ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Neonatal Brain Tissue (Mouse & Rat)	50 T	20 ~ 400 mg to be processed per time

Storage & Transportation

- ✧ Transported at 2℃ ~ 8℃.
- ✧ 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 all the enzyme reagents should be dissolved separately, mixed 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	HBSS Buffer (with $Ca^{2+}$and Mg^{2+})	PBS	RPMI 1640 or DMEM Medium
	Red Blood Cell Lysis Buffer (optional)		
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	70 μm Cell Strainer
Consumable	0.22 μm Syringe Filter (optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)	Constant Temperature Oscillator

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the powder of the enzyme A reagent with 11 mL HBSS Buffer (with $Ca^{2+}$and Mg^{2+}) in the 37℃ constant temperature oscillator, subpackage the solution and store at -25℃ ~ -15℃. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃ and it should avoid repeated freezing and thawing.

(2) Preparation of enzyme B solution: Dissolve the powder of the enzyme B reagent with 2.75 mL buffer B, subpackage the solution and store at -25℃ ~ -15℃. The enzyme solution can be stored stably for 6 months at -25℃ ~ -15℃ and it should avoid repeated freezing and thawing.

(3) Preparation of enzyme mixture:
Prepare the enzyme mixture according to the table below, and the enzyme mixture should be freshly prepared just before use. The enzyme mixture can be used for processing 20 ~ 400 mg neonatal brain tissue of mouse or rat. When processing neonatal brain tissue greater than 400 mg, the amount of tissue processing tube should be increased. If subsequent cell culture is required, the enzyme mixture needs to be sterile-filtered by the 0.22 μm syringe filter and the volume of the filtered mixture should be 2.05 mL in total.
⚠ Note: The enzyme A reagent solution should be incubated in the 37℃ constant temperature oscillator for 3 ~ 5 min to be fully dissolved before being used to prepare enzyme mixture.

Enzyme Mixture		
Buffer A 1800 μL	Enzyme A 200 μL	Enzyme B 50 μL

(4) Activation of enzyme mixture: Place the tissue processing tube containing enzyme mixture in the 37℃ constant temperature oscillator to incubate the enzyme mixture at 50 ~ 100 rpm for 25 ~ 30 min.

Mechanized Protocol

(1) After obtaining neonatal brain tissue of mouse or rat, put it in the petri dish containing PBS or RPMI 1640 or DMEM medium for temporary storage and the tissue should be submerged in the solution. Gently remove the blood streak on the tissue surface with the ophthalmic small curved forceps and cut the tissue into pieces of 2 ~ 4 mm with the ophthalmic scissors.

(2) Weigh target weight of the tissue pieces by the electronic balance scale and transfer the tissue pieces to the tissue processing tube containing enzyme mixture in step (4) of ***"Preparation"***.

(3) Tighten the tissue processing tube, invert it and mount it in the bushing of the single cell suspension dissociator with the heater.
⚠ Note: Make sure the sample is in the area where the rotor/stator is located.

(4) Run the program **M_NeoBrain_Heater_1**.

(5) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator. Blow and blend the cell suspension with the 1 mL pipette 8 ~ 10 times.

(6) Wet the 70 μm cell strainer with 1 mL PBS or RPMI 1640 or DMEM medium, filter the cell suspension through the wetted cell strainer, and collect the cell suspension to the 50 mL centrifuge tube.

(7) Rinse the tissue processing tube with 5 mL PBS or RPMI 1640 or DMEM medium, filter the suspension through the 70 μm cell strainer and collect it to the 50 mL centrifuge tube mentioned in step (6).

(8) Transfer the cell suspension collected in the 50 mL centrifuge tube in step (7) to the 15 mL centrifuge tube, centrifugate the cell suspension at 4℃, 300×g for 10 min and discard the supernatant completely.

(Optional) Removal of red blood cells

If it is necessary to remove the red blood cells, resuspend the cells collected in step (8) with 1 ~ 2 mL red

blood cell lysis buffer. Then, incubate the suspension on ice for 2 ~ 3 min, followed by resuspension with 10 mL PBS buffer or RPMI 1640 or DMEM medium. Centrifugate the cell suspension at 4°C, 300×g for 10 min and discard the supernatant completely.

(9) Resuspend the cell suspension with PBS or other buffer to required volume for subsequent experiment.

Manual Protocol

(1) After obtaining neonatal brain tissue of mouse or rat, put it in the petri dish containing PBS or RPMI 1640 or DMEM medium for temporary storage and the tissue should be submerged in the solution. Gently remove the blood streak on the tissue surface with the ophthalmic small curved forceps and cut the tissue into pieces of 2 ~ 4 mm with the ophthalmic scissors.

(2) Weigh target weight of the tissue pieces by the electronic balance scale and add the enzyme mixture incubated in step (4) of ***"Preparation"*** to the 50 mL centrifuge tube. Transfer the tissue pieces to the 50 mL centrifuge tube and tighten the tube cap.

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

(3) Place the 50 mL centrifuge tube in step (2) in the 37°C constant temperature oscillator, incubate the cell suspension at 50 rpm for 10 min and blow the tissue pieces 10 times by the 1 mL pipette, which the tip is cut off 0.5 cm. Then, repeat the incubation and blowing again.

(4) Follow the steps in ***"Mechanized Protocol"*** from step (6) to (9).

⚠ 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) 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) Processing 20 ~ 400 mg neonatal brain tissue of mouse and rat requires approximately 2.05 mL enzyme mixture for enzymatic digestion.

(4) The Kit has passed the transportation test, so the performance of the Kit is not affected though the ice pack equipped with the Kit has melted upon receipt.

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

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