High Activity Adult Brain Enzymatic Digestion Kit for Mouse&Rat (Trial Pack)

1. Product Information

Product Name	Product Model	Product Specification
High Activity Adult Brain Enzymatic Digestion Kit for Mouse&Rat	DHABE-10	10T

2. Product Description

High Activity Adult Brain Enzymatic Digestion Kit for Mouse&Rat can prepare single cell suspension gently, quickly and efficiently from Adult rat and mouse brain tissue (Adult rat and mouse P＞7, mainly focus on P9-12 Week adult mouse). This optimization scheme can help obtain as many single cell samples with high cell viability as possible, while maintaining the important surface epitopes of cells. The obtained single cell suspension can continue to be applied in downstream experiments such as cell sorting and primary cell culture, etc.

Main principle: By combining mechanical shearing and enzymatic digestion of extracellular matrix (to maintain the structural integrity of tissue), Adult rat and mouse brain tissue is prepared into single cell suspension. RWD single cell suspension preparation device mainly plays the role of mechanical dissociation, while the High Activity Adult Brain Enzymatic Digestion Kit for Mouse&Rat digests the tissue primarily through enzymolysis. After dissociation, the sample is filtered with a cell filter to remove the tissue residue in the sample, so that a single cell suspension is obtained. The resulted cells can be immediately used for subsequent experiments, such as primary cell culture, cell sorting, single cell sequencing, etc.

3. Product ingredients

4 bottles of reagents in total, including

1 bottle of Enzyme A reagent (solution)
1 bottle of Enzyme B reagent (solution)
1 bottles of Buffer A reagent (solution)
1 bottle of High efficiency debris removal reagent (solution)

4. Test capacity

Perform Adult rat and mouse brain tissue dissociation for 10 times,
Processing 20-500mg rat adult brain tissue; 20-300mg hippocampus tissue; 20-300mg spinal cord tissue each test.

5. Transport and storage

Transport in dry ice;
Store Enzyme A and Enzyme B in the kit at -25～-15℃, and the other four components (buffer A and High efficiency debris removal reagent) at 2～8℃, with a validity period of 6 months.

6. Reagent and Instrument Requirements

HBSS (containing Ca^{2+} and Mg^{2+}) or PBS solution
70 μm cell strainer
Constant temperature shaking water bath
DSC-400 Single Cell Suspension Dissociator (RWD)
Single cell tube (RWD)
HJ-400 Heater (Optional , RWD)

7. How to Use

7.1 Reagent Preparation

7.1.1 Prepare enzyme mixture

Prepare mix 1 according to the table below, and the enzyme mixture is freshly prepared just before use. The Enzyme mix 1 prepared below can be only used for at most 500 mg of brain tissue; 300mg of spinal cord tissue and hippocampus tissue. When working brain tissue is less than 500 mg, and the spinal cord and hippocampus tissue is less than 300mg, use the same volumes as indicated. When working with more than 500mg of brain tissue from adult rats or mouse, determine the weight and scale up all reagent volumes and total Enzyme mix 1 volumes accordingly. A maximum of 1000 mg brain tissue per tissue processing tube Tube can be processed.

Enzyme mix 1		
Enzyme A 100 μL	Buffer A 1850 μL	Enzyme B 50 μL

7.1.2 Activation of enzyme reagents

The prepared Enzyme mix1 was placed in a 37℃ constant temperature shaking water bath, rotate it continuously at 50 -100rpm and incubate for 25~30 min.

7.2 Gentle enzymolysis scheme for Adult rat and mouse brain tissue

（1） After stripping the Adult brain tissue, place and temporarily store the brain tissue in a petri dish containing HBSS (containing Ca^{2+} and Mg^{2+}) or PBS with solution overhead the brain tissue, and remove blood capillaries gently from brain tissue as much as possible by using small curved ophthalmic forceps.

（2） Weigh the Adult brain tissue. Add Enzyme mix 1 incubated in step 7.1.2 to a tissue processing tube. Then transfer the brain tissue to the tissue processing tube (the whole brain tissue needs to be cut 3 times with scissors into about 4 small pieces).

（3） Tighten the tissue processing tube, turn it upside down, and fit into the cannula of single cell suspension preparation device DSC-400 with HJ-400 Heating Jacket (Note: Make sure the sample material is in the area where the rotor/stator is located).

（4） Adult brain tissue and spinal cord tissue run program **M_ABrain_Heater_2**, hippocampus tissue program **M_ABrain_Heater_1**.

（5） After the program ends, remove the tissue processing tube from the single cell suspension preparation device DSC-400 with HJ-400 Heating Jacket, invert the tube, and short spin for 5-8 seconds to sink the sample tissue to the tube bottom. (Optional) To obtain more cells, blow the mixed cell suspension 8 times with a 1mL pipette.

(6) Wet a 70 μm cell strainer with 1 mL of PBS or HBSS (containing Ca^{2+} and Mg^{2+}), and filter the cell suspension sample with the wetted cell strainer, and collect the cell suspension in a 50 ml centrifuge tube.

(7) Rinse the tissue processing tube with 5 mL PBS or HBSS (containing Ca^{2+} and Mg^{2+}) and, after filtering through a 70 μm filter, collect it in the 50 mL centrifuge tube in step (6).

(8) Centrifuge the cell suspension at 300×g for 10 minutes and completely discard the supernatant.

(9) Resuspend cells to a desired volume with PBS or HBSS (containing Ca^{2+} and Mg^{2+}) for follow-up experiments.

7.3 Debris removal

（1） The weight range for processing is 20mg-1000mg, refer to the following table for debris removal processing:

Tissue weight	PBS	Debris removal solution	Overlay (PBS)	Reagent tube
20-100mg	1550μL	450μL	2mL	5 mL
101-500mg	3100μL	900μL	4mL	15 mL
501-1000mg	6200μL	1800μL	4mL	15 mL

（2） According to the tissue weight range, add the corresponding PBS to resuspend the cell pellet (the cell pellet obtained in 7.2 step (8) (Aspirate as much supernatant as possible and can not be shaken and resuspended)), and add the corresponding volume of debris efficient removal reagent (use a 1mL pipette Gently pipet 10 times to mix with the cell suspension) and the upper PBS volume (slowly add pre-cooled PBS along the wall of the centrifuge tube).

（3） Then, centrifuge the cell suspension at 3000 × g at 4°C, with a acceleration speed of 5 and a brake speed of 3 for 10 minutes(The different centrifuge the acceleration and brake can be appropriate reduced). After centrifugation, the solution is separated into three layers, and the top two layers are completely discarded, collect the lower layer of cells, add cold PBS solution to 10 mL (15 mL centrifuge tube) or 5 mL (5 mL centrifuge tube), invert up and down 3 times (do not shake and resuspend), centrifuge the cell suspension at 1000 × g for 10 minutes to wash, thoroughly discard the supernatant.

（4） Resuspend the cells to the desired volume with PBS or HBSS (containing Ca^{2+} and Mg^{2+}) for subsequent experiments.

7.4 Red blood cell removal (optional)

If erythrocyte removal is required, resuspend the cells treated in 7.3 step (3) with 1 mL of erythrocyte lysis solution (eg: Solarbio: #R1010 RBC lysis), then place on ice and incubate for 3-5 min, followed resuspend by 9 mL of HBSS (containing Ca^{2+} and Mg^{2+}) or PBS, centrifuge the cell suspension at 300× g for 10 minutes, completely discard the supernatant, and resuspend the cells in the appropriate buffer or medium by pipetting slowly up and down for subsequent experiments.

8 Precautions

(1) This kit is valid for 6 months, and RWD shall not guarantee the validity of expired products.

(2) When downstream cell culture is carried out after tissue dissociation, make sure that all operations are performed under sterile conditions.

(3) The enzyme A reagent stock solution needs to be incubated in a water bath at 37°C for 3-5 minutes, then completely dissolved and prepared into enzyme mix 1.

(4) About 2 mL of mixed enzyme solution is required for enzymatic digestion of each 20-500 mg of adult rat and mouse brain tissue.

(5) In the centrifugation step of removing debris, the speed of acceleration and brake is recommended to be 5 up and 3 down, mainly applicable to eppendorf and Thermo Fisher centrifuges. Other brands of centrifuges can refer to this speed for pre-experiment to determine a more appropriate speed of acceleration and deceleration.

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