High Activity Adult Brain Enzymatic Digestion Kit for Mouse&Rat

1 Product Information

Product Name	Product Model	Product Specification
High Activity Adult Brain Enzymatic Digestion Kit （Mouse&Rat）	DHABE-5003	50 T

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，hippocampus or cortex and spinal cord tissue (Adult rat and mouse P > 7, mainly focus on P9 ~ 12 Week). 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 and effectively remove myelin debris with the high-efficiency debris removal reagent included with the kit .So that a single cell suspension with cleaner background 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

6 bottles of reagents in total, including:

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

4 Test capacity

Perform Adult rat and mouse brain tissue dissociation for 50 times,

Processing 20 ~ 500 mg rat adult brain tissue, 20 ~ 300 mg hippocampus tissue, 20 ~ 300 mg cortex tissue, 20 ~ 300 mg spinal cord tissue each test.

5 Transport and storage

Transport in ice packs at 2 ~ 8°C;

Store one component (Enzyme B) in the kit at -25 ~ -15 °C, and the other components (Enzyme A, buffer A , buffer B and High efficiency debris removal reagent) at 2 ~ 8 °C, with a validity period of 12 months.

6 Reagent and Instrument Requirements

HBSS (containing Ca^{2+} and Mg^{2+}) or PBS solution;
70 μm cell strainer;
Constant temperature oscillator;
DSC-400 Single Cell Suspension Dissociator (RWD);
Tissue processing tube* (RWD) ;
HJ-400 Heater (Optional, RWD).

7 How to Use

7.1 Reagent Preparation

7.1.1 Dissolve enzyme dry powder

1) Prepare Enzyme A solution: dissolve the powder in the Enzyme A reagent bottle with 5.5 mL HBSS (containing Ca^{2+} and Mg^{2+}) at 37 °C water bath and mix well. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.
2) Prepare enzyme B solution: dissolve the powder in the enzyme B reagent bottle with 2.75 mL Buffer B. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

7.1.2 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 used for 20 ~ 500 mg brain tissue, 20 ~ 300 mg hippocampus tissue, cortex tissue and spinal cord tissue . When working with more than 500 mg 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 and 300 mg hippocampus tissue, cortex tissue or spinal cord tissue per tissue processing tube can be processed. If more tissue is to be processed, the number of tissue processing tubes needs to be increased. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered (e.g., filtration with a 0.22 μm syringe filter). After filtration, the total volume of enzyme mixture should be 2 mL.

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

7.1.3 Activation of enzyme reagents

The prepared Enzyme mix1 was placed in a 37 °C constant temperature oscillator, rotate it continuously at 50 ~ 100 rpm 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, hippocampus tissue (A mice has two pieces of hippocampus), cortex tissue or spinal cord 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 the above tissue as much as possible by using small curved ophthalmic forceps.
2) Weigh the Adult brain tissue. Add Enzyme mix 1 incubated in step 7.1.3 to a tissue processing tube. Then transfer the brain tissue to the tissue processing tube (the whole brain tissue needs to be cut into about 4 small pieces with scissors, a piece of hippocampus tissue needs to be cut into 2 small pieces, a cortical tissue needs to be cut into 4 small pieces, and the spinal cord tissue needs to be cut into 0.5 cm length 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 Heater (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 Heater, invert the tube, and short spin for 7 seconds or centrifuge at 300g for 15s 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((Aspirate the supernatant with a pipette in this step)).
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 20 mg ~ 1000 mg, refer to the following table for debris removal processing:

Tissue weight	PBS	Debris removal solution	Overlay (PBS)	Reagent tube
20 ~ 100 mg	1550 μL	450 μL	2 mL	5 mL or 15 mL
101 ~ 500 mg	3100 μL	900 μL	4 mL	15 mL
501 ~ 1000 mg	6200 μL	1800 μL	4 mL	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 1 mL 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 1×erythrocyte lysis solution (eg:Biolegend: #420301 RBC lysis), then place on ice and incubate for 2 ~ 3 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 12 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) Each tissue processing tube can process a maximum of 1000 mg of adult rat brain tissue and 300 mg of spinal cord tissue，cortex tissue and hippocampus tissue, and the number of cells per unit weight obtained will be reduced when processing the weight of brain tissue more than 500 mg with single tissue processing tube.
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.
6) Due to weather conditions, the performance of the kit will not be affected even if the ice packs are dissolved when the kit is received. The kit has been tested for transportation.

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

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