High Activity Adult Brain Enzymatic Digestion Kit Instruction

Product Information

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

Description

High Activity Adult Brain Enzymatic Digestion Kit (Mouse and Rat) combines mechanical shearing and enzymatic digestion of the extracellular matrix (while maintaining the integrity of tissue structure) to gently, quickly, and efficiently prepare adult rat and mouse brain tissue, hippocampus, cortex, and spinal cord tissue (Adult rat and mouse P>7, mainly focus on P9 ~ 12 Week) into a single cell suspension. This optimized protocol can help obtain as many single cell samples with high cell viability as possible, while preserving the important surface antigenic epitopes of the cells.

This Kit can be used in conjunction with the RWD Single Cell Suspension Dissociator, which primarily plays a role in mechanical dissociation, while the Kit mainly digests the tissue through enzymatic dissociation. After dissociation, a cell strainer is used to filter the sample to remove tissue debris, and the Kit also includes a reagent for the efficient removal of myelin debris from the brain tissue. This product can also be used to obtain a single cell suspension directly following a manual enzymatic digestion protocol.

The single cell suspensions gained from the Kit can be used for subsequent experiments, such as primary cell culture, cell sorting, and single cell sequencing.

Components

Product Name	Components	Specification	Storage Condition
High Activity Adult Brain Enzymatic Digestion Kit (Mouse and Rat)	Enzyme A reagent (powder)	1 vial	2 ~ 8℃
	Enzyme B reagent (powder)	1 vial	-25 ~ -15℃
	Enzyme C reagent (powder)	1 vial	2 ~ 8℃
	Buffer A (solution)	2 vial	2 ~ 8℃
	Buffer B (solution)	1 vial	2 ~ 8℃
	Debris Removal Kit (solution)	1 vial	2 ~ 8℃

Test Capacity

Recommendation for single process:

Tissue Type	Capacity	Initial Sample Dosage
Rat adult brain tissue	50 T	20 ~ 500 mg to be processed per time
Hippocampus tissue	50 T	20 ~ 300 mg to be processed per time
Cortex tissue	50 T	20 ~ 300 mg to be processed per time
Spinal cord tissue	50 T	20 ~ 300 mg to be processed per time

Storage & Transportation

- ✧ Transported at 2 ~ 8℃.
- ✧ Enzyme B reagent should be stored at -25 ~ -15℃ and the other components at 2 ~ 8℃.
- ✧ It is recommended to dissolve the enzyme reagent and mix it well and store it in separate packages. Avoid repeated freezing, thawing and shake.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagent & Instrument

Reagent	HBSS (with Ca^{2+} and Mg^{2+})	PBS	Red Blood Cell Lysis Buffer (Optional)
Consumable	Tissue Processing Tube (RWD)	Heater (RWD # HJ-400)	70 μm Cell Strainer
	0.22 μm Syringe Filter (Optional)		
Instrument	Single Cell Suspension Dissociator (RWD)	High Speed Refrigerated Centrifuge	Constant Temperature Oscillator

Operation

Preparation

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

(2) Preparation of Enzyme B solution: Dissolve the powder in the Enzyme B reagent vial containing 2.75 mL Buffer B. After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C and avoid repeated freezing, thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

(3) Preparation of Enzyme C solution: Dissolve the powder in the Enzyme C reagent vial containing 2.75 mL HBSS (with Ca^{2+} and Mg^{2+}). After dissolution, sub-pack the solution directly, followed by frozen storage at -25 ~ -15°C and avoid repeated freezing, thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃.

(4) Preparation of Enzyme mix 1: Refer 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.

⚠ Note: The Enzyme A reagent storage solution needs to be incubated in a 37°C water bath for 3 ~ 5 minutes until it is completely dissolved before preparing the Enzyme mix 1.

Enzyme mix 1			
Enzyme A 100 μL	Buffer A 1800 μL	Enzyme B 50 μL	Enzyme C 50 μL

⚠ Note: If you need to test the CD8a and Tmem119 epitopes, please prepare the enzyme mix 2 according to the table below. This enzyme mixture should be prepared and used immediately. It provides good protection for the epitopes but may slightly reduce the yield.

Enzyme mix 2		
Buffer A 1850 μL	Enzyme B 50 μL	Enzyme C 100 μL

(5) Reagent Activation of Enzyme mix 1: Place the Enzyme mix1 in a 37 °C constant temperature oscillator, rotate it continuously at 50 ~ 100 rpm and incubate for 25 ~ 30 min.

⚠ Note: Enzyme mix 2 can be used directly without the need for water bath incubation.

Mechanized Protocol

(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

(with 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 appropriate ophthalmic forceps.

(2) Weigh the adult brain tissue, hippocampus tissue, cortex tissue or spinal cord tissue. Add Enzyme mix 1 incubated in step (5) in ***Preparation*** to a tissue processing tube. Then transfer the brain tissue to the tissue processing tube (the entire 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 pieces of 0.5 cm).

(3) Tighten the tissue processing tube, turn it upside down, and fit into the cannula of single cell suspension dissociator with heater.

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

(4) For adult brain tissue, cortex tissue and spinal cord tissue, run program **M_ABrain_Heater_2**; for hippocampus tissue, run program **M_ABrain_Heater_1**.

(5) After the program ends, remove the tissue processing tube from the single cell suspension dissociator, invert the tube, and short spin for 7 seconds or centrifuge at 300×g 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 1 mL pipette.

(6) Wet a 70 μm cell strainer with 1 mL of PBS or HBSS (with 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 10 mL PBS or HBSS (with Ca^{2+} and Mg^{2+}), 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) Use High Efficiency Debris Removal Kit (RWD: #DHDR-5006) to remove the debris.

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

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

(b) According to the tissue weight range, add the corresponding PBS to resuspend the cell pellet which obtained in 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 to gently pipet 10 times to mix with the cell suspension) and the upper PBS (slowly add pre-cooled PBS along the wall of the centrifuge tube).

(c) Then, centrifuge the cell suspension at 3000×g at 4°C, with an acceleration speed of 9 and a deceleration speed of 3 for 10 minutes (The acceleration and deceleration of different centrifuge can be appropriately 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 (use a 15 mL centrifuge tube) or 5 mL (use a 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.

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

(Optional) If erythrocyte removal is required, it is recommended to adopt the following method:

Resuspend the cells treated in step (9) with 1 mL of 1×red blood cell lysis buffer, then place on ice and incubate for 2 ~ 3 min. Then, resuspend by 9 mL of HBSS (with 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 for subsequent experiments.

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

Manual Protocol

(1) After stripping the adult brain tissue, hippocampus tissue (each mouse has two pieces of hippocampus), cortex tissue, or spinal cord tissue, place them temporarily in a petri dish containing HBSS (with Ca^{2+} and Mg^{2+}) or PBS, ensuring the solution covers the tissue. Use appropriate ophthalmic forceps to gently remove as much of the blood vessels from the surface of the tissue as possible.

(2) Weigh the adult brain tissue, hippocampus tissue, cortex tissue, or spinal cord tissue. First, add the mix 1 or mix 2 from the step (5) in ***Preparation*** of the experimental procedure into a 50 mL centrifuge tube. Then, transfer the corresponding tissue into the 50 mL centrifuge tube (the entire brain tissue should be cut into about 8 small pieces with scissors, one piece of hippocampus tissue should be cut into 4 halves, a piece of cortex tissue should be cut into 8 small pieces, and the spinal cord tissue should be cut into 0.2 cm length segments), and securely close the centrifuge tube cap.

(3) Place the 50 mL centrifuge tube from step (2) into a 37°C oscillator and incubate at 100 rpm for 15 minutes. After incubation, use a 1 mL pipette with the first 0.5 cm tip cut off to pipette up and down 20 times to blow the tissue. Then, place it back into the 37°C oscillator and incubate at 100 rpm for another 15 minutes. After the second incubation, pipette up and down 20 times again with the 1 mL pipette and the modified tip.

(4) Proceed with the subsequent operations according to steps (6) to (10) of the automated enzymatic digestion protocol.

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) 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 in a single tissue processing tube.

(4) In the centrifugation step of removing debris, the speed of acceleration and deceleration is recommended to be 9 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 the appropriate speed of acceleration and deceleration.

(5) Due to the weather, the performance of the Kit will not be affected even if the ice packs are dissolved when the Kit is received. The Kit has passed the transportation test.

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

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