High Activity Neonatal Brain Enzymatic Digestion Kit for Mouse&Rat

1 Product Information

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

2 Product Description

High Activity Neonatal Brain Enzymatic Digestion Kit for Mouse&Rat can prepare single cell suspension gently, quickly and efficiently from neonatal rat and mouse brain tissue (fetal rat brain or neonatal brain P≤7). 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), neonatal 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 Neonatal 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

5 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).

4 Test capacity

Perform neonatal rat and mouse brain tissue dissociation for 50 times, processing 20 ~ 400 mg brain tissue each time.

5 Transport and storage

Transport in 2 ~ 8°C ice bags;

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

6 Reagent and Instrument Requirements

HBSS (without Ca^{2+} and Mg^{2+}) or PBS solution;
EBSS (with Ca^{2+} and Mg^{2+}, Solarbio H2020);
70 μm cell strainer;
Constant temperature shaking water bath;
DSC-400 Single Cell Suspension Dissociator (RWD);
Tissue processing tube* (RWD);
(Optional) HJ-400 Heater (RWD).

7 How to Use

7.1 Reagent Preparation

1) Prepare enzyme A solution: dissolve the powder in the enzyme A reagent bottle with 11 ml EBSS (containing Ca^{2+} and Mg^{2+}) at 37°C water bath and mix well. After dissolution, sub-pack the solution directly, and avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months.
2) Prepare enzyme B solution: dissolve the powder in the enzyme B reagent bottle with 2.75 ml Buffer. After dissolution, sub-pack the solution directly, and avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can be stored stably for 6 months.
3) Prepare mix 1 and mix 2 according to the table below, and the enzyme mixture is freshly prepared just before use.

Enzyme mix 1		Enzyme mix 2
Enzyme A 200 μl	Buffer A 1800 μl	Enzyme B 50 μl

7.2 Gentle enzymolysis scheme for neonatal rat and mouse brain tissue

7.2.1 Using the single cell suspension preparation device DSC-400 with HJ-400 Heating Jacket

1) After stripping the neonatal brain tissue, place and temporarily store the brain tissue in a petri dish containing HBSS or PBS (without Ca^{2+} and Mg^{2+}) 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 neonatal brain tissue. The enzyme mix 1 prepared in **7.1** above can be only used for at most 400 mg of brain tissue.
3) Add 2,000 μL of enzyme mix 1 to a tissue processing tube, place the tube in a constant temperature shaking water bath, rotate it continuously at 50 rpm, and incubate at 37°C for 30 min.
4) After the incubation, transfer the brain tissue and 50 μL of enzyme mix 2 to a tissue processing tube containing 2,000 μL

of enzyme mix 1 in step 3).

5) Tighten the tissue processing tube, turn it upside down, and fit into the cannula of single cell suspension preparation device DSC-400 (Note: Make sure the sample material is in the area where the rotor/stator is located).
6) Run the program **M_NeoBrain_Heater_1**.
7) After the program ends, proceed with step 17) in **7.2.2** until the operation finishes.

7.2.2 Using single cell suspension preparation device DSC-400 only

1) After stripping the neonatal brain tissue, place and temporarily store the brain tissue in a petri dish containing HBSS (without Ca^{2+} and Mg^{2+}) 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 neonatal brain tissue. The enzyme mix 1 prepared in **7.1** above can be only used for at most 400 mg of brain tissue.
3) Add 2,000 µL of enzyme mix 1 to a tissue processing tube, place the tube in a constant temperature shaking water bath, rotate it continuously at 50 rpm, and incubate at 37°C for 30 min.
4) After the incubation, transfer the weighted neonatal brain tissue and 50 µL of enzyme mix 2 to a tissue processing tube containing 2,000 µL of enzyme mix 1 in step 3).
5) Tighten the tissue processing tube, turn it upside down, and fit into the cannula of single cell suspension preparation device DSC-400 (Note: Make sure the sample material is in the area where the rotor/stator is located).
6) Run the program **Mouse_Brain_1**.
7) After the program ends, remove the tissue processing tube from the single cell suspension preparation device DSC-400.
8) Place the tissue processing tube in a constant temperature shaking water bath, rotate continuously at 50 rpm, and incubate at 37°C for 15 minutes. Always keeps the tissue processing tube upside down to avoid waste of tissue remaining on the tube wall.
9) After incubation, invert and install the tissue processing tube into the cannula of single cell suspension preparation device DSC-400. (Note: Make sure the sample material is in the area where the rotor/stator is located).
10) Run the program **Mouse_Brain_2**.
11) After the program ends, remove the tissue processing tube from the single cell suspension preparation device DSC-400.
12) Place the tissue processing tube in a constant temperature shaking water bath, rotate continuously at 50 rpm, and incubate at 37°C for 10 minutes. Always keeps the tissue processing tube upside down to avoid waste of tissue remaining on the tube wall.
13) After incubation, invert and install the tissue processing tube into the cannula of single cell suspension preparation device DSC-400. (Note: Make sure the sample material is in the area where the rotor/stator is located).
14) Run the program **Mouse_Brain_3**.
15) After the program ends, remove the tissue processing tube from the single cell suspension preparation device DSC-400.
16) Place the tissue processing tube in a constant temperature shaking water bath, rotate continuously at 50 rpm, and incubate at 37°C for 10 minutes. Always keeps the tissue processing tube upside down to avoid waste of tissue remaining on the tube wall.
17) Afterwards, remove the tissue processing tube from the single cell suspension preparation device DSC-400, invert the tube, and shake it for 3 seconds to sink the sample tissue to the tube bottom.
18) 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.
19) 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 18).
20) Transfer the cell suspension from the 50 mL centrifuge tube in step 19) to a 15 mL centrifuge tube, centrifuge the cell suspension at 300×g for 10 minutes and completely discard the supernatant.
21) Resuspend cells to a desired volume with PBS or HBSS (containing Ca^{2+} and Mg^{2+}) for follow-up 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) About 2 mL of mixed enzyme solution is required for enzymatic digestion of each 20 ~ 400 mg of neonatal rat and mouse brain tissue.
4) 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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