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

## 1. Product Information

| Product Name | Product Model | Product Specification |
|---|---|---|
| High Activity Neonatal Brain Enzymatic Digestion Kit for Mouse&Rat | DHNBE-10 | 10T |

## 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

3 bottles of reagents in total, including

1 bottle of enzyme A reagent (powder)
1 bottle of enzyme B reagent (powder)
1 bottles of Buffer A reagent (solution)

## 4. Test capacity

Perform neonatal rat and mouse brain tissue dissociation for 10 times, processing 20-400 mg brain tissue each time.

## 5. Transport and storage

Transport in 2 ~ 8℃ ice bags;

Store one component (enzyme B) in the kit at -25～-15℃, and the other three components (enzyme A, buffer A and buffer B) at 2～8℃, 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)
Single cell tube (RWD)
(Optional) HJ-400 Heater (RWD)

## 7. How to Use

### 7.1 Reagent Preparation

**7.1.1** Prepare enzyme A solution: Before the experiment, the solution should be dissolved in 37℃ water bath and shaken evenly until the solution is clarified.

**7.1.2** 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 7.1.3 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 ℃ 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 ℃ 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 ℃ 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 ℃ 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 ℃ 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 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) 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) Before the experiment, the enzyme A and enzyme B reagents should be centrifuged instantly for 10s to avoid waste caused by more solution sticking to the wall of the tube.



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