# High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) Instructions

## 1 Product Information

| Product Name | Model | Specification |
|---|---|---|
| High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) | DHIE-5007 | 50 T |

## 2 Description

The High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) allows for the gentle, rapid, and efficient preparation of adult mouse (6 ~ 10 weeks) intestinal (the small intestine) tissue into a single cell suspension. This optimized protocol helps to obtain as many highly-viable single cell samples 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.

**Main principle**: Mouse intestinal tissue is prepared as a single cell suspension by a combination of mechanical shearing and enzymatic digestions of the extracellular matrix to keep the tissue structurally intact. The RWD Life Science Single Cell Suspension Dissociator is chiefly used for mechanical dissociation, while the High Activity Intestine Tissue Enzymatic Digestion Kit (Mouse) mainly digests the tissues through enzymatic hydrolysis. After dissociation, the sample is filtered with a cell strainer to remove tissue residues to obtain a single cell suspension, and the obtained cells could be used immediately for subsequent experiments, such as primary cell culture, cell sorting, single cell sequencing, etc.

## 3 Components

8 bottles of reagents in total, including:

1 bottle of Enzyme A (powder);
1 bottle of Enzyme B (powder);
1 bottle of Enzyme C (powder);
1 bottle of Buffer B (solution);
1 bottle of Buffer C (solution);
1 bottle of Buffer D (solution);
1 bottle of Buffer E (solution);
1 bottle of Buffer H (solution).

## 4 Test Capacity

Perform high activity intestine tissue dissociation for 50 times, processing 200 ~ 600 mg each test.

## 5 Transport and Storage

Transport in ice pack at 2 ~ 8℃;

Store Enzyme C powder, Buffer D and Buffer E components at -25 ~ -15℃ and Enzyme A powder, Enzyme B powder, Buffer B, Buffer C and Buffer H components at 2 ~ 8℃, with validity period of 12 months.

## 6 Reagent and Instrument Requirements

DSC-400 single cell suspension dissociator (RWD);
Heater (RWD);
Tissue processing tube*(RWD);
(Recommended and optional) D-Hanks (Without $Ca^{2+}$ and $Mg^{2+}$) (Solarbio: H1040);
(Recommended and optional) Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) (Solarbio: H1020);
(Recommended and optional) Red blood cell lysis buffer (Solarbio: R1010);
Constant temperature oscillator;
100 μm cell strainer;
Fetal bovine serum (FBS);

PBS;

PB (PBS with 0.5% BSA).

## 7 Method for use

### 7.1 Preparing and Aliquoting Reagents

1) Fully dissolve Enzyme A powder with 5.5 mL Hanks (containing $Ca^{2+}$ and $Mg^{2+}$);
2) Fully dissolve Enzyme B powder with 1.4 mL Buffer B;
3) Fully dissolve Enzyme C powder with 1.4 mL Buffer C.

Dissolve and aliquot the enzyme reagents above and store them at -25 ~ -15℃ to avoid repeated freezing and thawing and vibrate agitation. The enzyme solution can bestored stably for 6 months at -25 ~ -15℃;

Similarly, aliquot and freeze Buffer D and Buffer E as needed to avoid repeated freezing and thawing during use, and store them at -25 ~ -15℃;

Other reagents can be stored at 2 ~ 8℃.

### 7.2 Preparation of Cleaning Solution

| Preparation Volume | Preparation Process | PH Adjustment |
|---|---|---|
| 20 mL | 17.8 mL D-Hanks (Without $Ca^{2+}$ and $Mg^{2+}$) + 1 mL Fetal bovine serum (FBS) + 20 μL Buffer D + 1 mL Buffer E + 200 μL Buffer H | Add 25 μL 4M NaOH to a PH of 7.2 ~ 7.4 |

### 7.3 Preparation of Enzymatic Hydrolysis System

Prepare the corresponding enzyme mixture in the tissue processing tube. If subsequent cell culture is required, the enzyme mixture should be sterile-filtered with a 0.22 μm syringe filter. After filtration, the total volume of enzyme mixture should be 2.5 mL.

| Sample Range | Enzyme Mixture | Enzyme A | Enzyme B | Enzyme C |
|---|---|---|---|---|
| 200 mg ~ 600 mg | 2.12 mL Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) + 20 μL Buffer H + 210 μL FBS | 100 μL | 25 μL | 25 μL |

### 7.4 Protocol for Gentle Enzymolysis of Tissue

#### 7.4.1 Application of DSC-400 Single Cell Suspension Dissociator (HJ-400 Heater Included)

1) Take the intestines of 6 ~ 10 weeks old ICR or C57 mice (mainly from the small intestine), and place them in Petri dishes containing cold PBS;
2) Remove excess fat, lymphoid tissue, and blood streaks, and longitudinally cut the intestinal tissue to remove fecal impurities. Rinse 3 ~ 5 times with cold PBS until no obvious impurities are visible, dry the intestinal surface using the device, and then transversely cut the intestinal tract into pieces about 0.5 ~ 1 cm long;

   ⚠ **Note: The tissue should be cut as short as possible; if the tissue block is too long, it may get stuck in the tissue processing tube and result in the existence of tissue residue.**
3) Weigh the tissue according to the sample range, transfer the tissue block to a 50 mL centrifuge tube containing 20 mL PBS, and then place the tube on a vortex shaker to shake and wash for 30 seconds at 1000 ~ 1500 rpm. Filter and remove the PBS with a 100 μm cell strainer;
4) Transfer the tissue block from the cell strainer to a 50 mL centrifuge tube containing 20 mL of the cleaning solution, and wash the sample tissue in a constant temperature oscillatorat 37°C at 150 rpm for 30 minutes with the centrifuge tube tilted so that the tissue block in it is well shaken;

   ⚠ **Note: Also transfer the enzyme mixture to the tissue processing tube and preheat at 37°C for 30 minutes.**
5) After washing, filter the tissue sample using a 100 μm cell strainer, and collect the tissue on the cell strainer into a 50 mL centrifuge tube containing 20 mL of PBS. Shake and wash 10 times, and filter using the 100 μm cell strainer again to discard the PBS;

6) Repeat step 5) once;
7) Transfer the tissue sample from the cell strainer to a tissue processing tube containing the enzyme mixture;
8) Add 100 μL of Enzyme A, 25 μL of Enzyme B, and 25 μL of Enzyme C to the above-mentioned tissue processing tube containing the tissue sample, tighten the tube, and mix them gently and evenly;
9) Install the tube upside down into the sleeve of the DSC-400 single cell suspension dissociator and install the heater;

   ⚠ **Note: Make sure the sample material is in the area where the rotor/stator is located.**
10) Run "M_Intestine_Heater_1";
11) At the end of the program, remove the tissue processing tube from the single cell suspension dissociator, rinse the 100 μm cell strainer with PBS, filter the cell suspension sample using the wetted cell strainer, and collect the cell suspension into a 50 mL centrifuge tube;
12) Wash the tissue processing tube with 10 mL PBS, filter through a 100 μm cell strainer, and collect into a 50 mL centrifuge tube from step 11);
13) Centrifuge the cell suspension at 300×g for 10 minutes at room temperature and discard the supernatant completely;
14) Resuspend the cells to the desired volume with an appropriate amount of PB for further applications. The resuspended cells are used for primary cell culture or flow cytometry;

    ⚠ **Note: if flocculent impurities appear after resuspension, use a 40 μm cell strainer to filter again.**
15) (Optional) If red blood cell lysis is required, refer to the recommended method.
    (Recommended) Resuspend the cell pellet obtained after step 13) with 2 mL of the red blood cells lysis solution (Solarbio: R1010), and then incubate on ice for 1 ~ 3 minutes, followed by termination with 9 mL of RPMI 1640 or DMEM. Centrifuge the cell suspension at 300×g for 10 minutes and discard the supernatant completely.

## 8 Precautions

1) The shelf life of this kit is 12 months, and RWD will not guarantee the validity of expired products;
2) When culturing downstream cells after tissue dissociation, make sure that all operations are performed under sterile conditions;
3) Steps 5) and 6) of **7.4.1** of the enzymatic hydrolysis protocol significantly affect the experimental results and thus should not be omitted;
4) Buffer D has low toxicity. Wear gloves to avoid any contact with the skin during use. If this happens, repeatedly wash with clean water or soapy water as soon as possible;
5) 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 reagents can be stored at 37°C for 2 days.
   *Note: The tissue processing tubes of RWD are not available in the USA market.

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