# High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse and Rat) Instructions

## 1 Product Information

| Product Name | Model | Specification |
|---|---|---|
| High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse and Rat) | DHAE-5010 | 50 T |

## 2 Description

High Activity Adipose Tissue Enzymatic Digestion Kit (Mouse and Rat) can prepare the adipose tissue into a single cell suspension in a gentle, quick, and efficient manner. This optimization scheme can help obtain as many highly-viable single cell samples as possible while maintaining the important surface epitopes of cells. The prepared single cell suspension, where the adipocytes are immature, is mainly used for downstream experiments such as SVF (Stromal Vascular Fraction) cell culture or cell sorting.

**Main principle:** The adipose 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 Adipose Tissue Enzymatic Digestion Kit (Mouse and Rat) mainly digests the tissue through enzymatic hydrolysis. After dissociation, the sample is filtered with a cell strainer to remove the tissue residues in the sample, so that a single cell suspension is obtained. The resulting SVF cells can be used immediately for subsequent experiments, such as primary cell culture, cell sorting, single cell sequencing, etc.

## 3 Components

5 bottles of reagents in total, including:

1 bottle of Enzyme A reagent (lyophilized powder);
1 bottle of Enzyme B reagent (lyophilized powder);
1 bottle of Enzyme C reagent (lyophilized powder);
1 bottle of Buffer B reagent;
1 bottle of Buffer C reagent.

## 4 Test Capacity

The kit can at least dissociate the adipose tissue 50 times within the sample range, processing 0.1 ~ 1.0 g (not included) of eWAT or 0.1 ~ 0.5 g (not included) of BAT or iWAT each time. The added volume of enzyme mix varies according to the tissue types processed. Refer to **table 4-1** for details.

Table 4−1

| Sample types | Enzyme Mixture |
|---|---|
| eWAT 0.1 ~ 1.0 g (not included) | 2.4 mL Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) + 75 μL Enzyme A + 75 μL Enzyme B + 12.5 μL Enzyme C |
| BAT or iWAT 0.1 ~ 0.5 g (not included) | 2.3 mL Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) + 100 μL Enzyme A + 100 μL Enzyme B + 25 μL Enzyme C |

The prepared enzyme mix should be sterile-filtered with a 0.22 μm filter if primary cell culture will be performed subsequently. The total volume of the enzyme mix after filtration should be 2.5 mL. If over 1.0 g of eWAT or over 500 mg of BAT or iWAT needs to be digested, it is recommended that adipose tissue is allocated to multiple tissue processing tubes as part of the experiments.

## 5 Transport and Storage

Ship the kit with an ice pack at 2℃ ~ 8℃;

Store the kit by components upon receipt. Store the Enzyme C reagent at -25℃ to -15℃, with the remaining reagents stored at 2℃ ~ 8℃. The shelf life of all 5 reagents is 12 months.

## 6 Reagent and Instrument Requirements

Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) (Solarbio: #H1020) or RPMI 1640;
100 μm cell strainer;
(Optional) 40 μm cell strainer;
DSC-400 / DSC-800 single cell suspension dissociator (RWD);
Constant temperature oscillator;
Tissue processing tube* (RWD);
HJ-400 heater (RWD);

(Optional) Red blood cell lysis buffer (Solarbio: R1010);
(Optional) 0.22 μm syringe filter.

## 7 Method for Use

### 7.1 Reagent Preparation

1) Preparation of Enzyme A solution: Add 6 mL Hanks (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640 to the vial containing Enzyme A lyophilized powder and mix them well;
2) Preparation of Enzyme B solution: Add 6 mL Buffer B to the vial containing Enzyme B lyophilized powder and mix them well;
3) Preparation of Enzyme C solution: Add 1.5 mL Buffer C to the vial containing Enzyme C lyophilized powder and mix them well.

15 mL centrifuge tubes can be selected to help dissolve Enzyme A and Enzyme B solutions. To avoid repeated freezing and thawing, store all the dissolved components separately in a proper amount of tubes.

When stored at -25℃ to -15℃, the dissolved components can be stable for 6 months.

### 7.2 Solutions for High Activity Adipose Tissue Enzymatic Digestion

7.2.1 Application of DSC-400 / DSC-800 Single Cell Suspension Dissociator (HJ-400 Heater Included)

1) Following the description in **table 4-1**, add Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) and components of enzymatic reagents to a tissue processing tube to prepare the enzyme mix.
   ⚠ Note: If Hanks buffer is not available, RPMI 1640 basic culture medium can be used temporarily as an alternative.
2) Take the adipose tissue and repeatedly rinse it with PBS until clean. Then store the tissue block temporarily in a container with Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640. Cut the tissue block into 2 ~ 4 mm pieces. Weigh the pieces for targeted weight using an electronic balance.
   ⚠ **Note: The adipose tissue should be taken upon immediate use.** Lymph nodes, blood stains, and redundant connective tissue septa that may be contained in each adipose tissue should be removed as much as possible.
3) Transfer the pieces of adipose tissue into a tissue processing tube containing enzyme mix.
4) Tighten the tissue processing tube. Invert and install the tube into the sleeve of the DSC-400 / DSC-800 single cell suspension dissociator.
   ⚠ Note: Make sure the sample material is in the area where the rotor/stator is located.
5) Run the program **M-Adipose-Heater-1**.
6) When the program runs to the end, remove the tissue processing tube from the DSC-400/DSC-800 Single Cell Suspension Dissociator (with heating jackets), and continue with **Step 10** in **7.2.2** until the end.

7.2.2 Application of DSC-400 / DSC-800 Single Cell Suspension Dissociator Only

1) Following the description in **table 4-1**, add Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) and components of enzymatic reagents to a tissue processing tube to prepare the enzyme mix.
   ⚠ Note: If Hanks buffer is not available, RPMI 1640 basic culture medium can be used temporarily as an alternative.
2) Take the adipose tissue and repeatedly rinse it with PBS until clean. Then store the tissue block temporarily in a container with Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640. Cut the tissue block into 2 ~ 4 mm pieces. Weigh the pieces for targeted weight using an electronic balance.
   ⚠ **Note: The adipose tissue should be taken upon immediate use.** Lymph nodes, blood stains, and redundant connective tissue septa that may be contained in each adipose tissue should be removed as much as possible.
3) Transfer the pieces of adipose tissue into a tissue processing tube containing enzyme mix.
4) Tight the cover of the tissue processing tube. Place the tissue processing tube upside down into a constant temperature oscillator, and perform continuous rotation and incubation at 37°C at 100 rpm for 20 minutes.
   ⚠ Note: Always keep the tissue processing tube upside down so that no residual tissue will adhere to the tube wall.
5) After incubation, insert the tissue processing tube upside down into the DSC-400/DSC-800 Single Cell Suspension Dissociator.
   ⚠ Note: Make sure the sample material is in the area where the rotor/stator is located.

6) Run the program **M-Adipose-01**. When the program runs to the end, remove the tissue processing tube from the single cell suspension dissociator.
7) Place the tissue processing tube upside down into a constant temperature oscillator, and perform continuous rotation and incubation at 37°C at 100 rpm for 20 minutes.
   ⚠ Note: Always keep the tissue processing tube upside down so that no residual tissue will adhere to the tube wall.
8) After incubation, insert the tissue processing tube upside down into the DSC-400/DSC-800 Single Cell Suspension Dissociator.
   ⚠ Note: Make sure the sample material is in the area where the rotor/stator is located.
9) Rerun the program **M-Adipose-01**. When the program runs to the end, remove the tissue processing tube from the single cell suspension dissociator.
10) Rinse the 100 μm cell strainer with 1 mL Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640 culture medium, filter the cell suspension sample using the wetted 100 μm cell strainer, and collect the cell suspension into a 50 mL centrifuge tube.
11) Then rinse the tissue processing tube with 20 mL Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640. Filter the resulting solution with the 100 μm strainer and collect it into the 50 mL centrifuge tube mentioned in Step 10.
   ⚠ Note: If the filtration of BAT leaves many residues, a pipette tip that sucked the buffer or RPMI 1640 can be used to help rinse the strainer.
12) Centrifuge the cell suspension for 10 minutes at 300×g. Carefully suck and discard all the supernate (including the uppermost layer of floating grease, the secondary layer of milky mature adipocytes, and the intervening mixture). Take care not to decant it directly to avoid loss of the SVF cell precipitates at the bottom.
13) Resuspend the cells to the desired volume with an appropriate amount of buffer or culture medium for further experiment applications. If floccules exist in the cell suspension of iWAT, the 40 μm cell strainer can be selected to filter the suspension directly before use.
14) (Optional) To remove the red cells, resuspend the cell precipitates mentioned in Step 12 by using 1 mL of red cell lysis solution (Solarbio: #R1010). Incubate the resulting suspension on ice for about 2 minutes, then terminate the incubation by adding over 6 mL RPMI 1640. Centrifuge the cell suspension at 300×g for 10 minutes. Completely discard the supernate for another resuspension.

## 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 always under sterile conditions.
3) Epididymal white adipose tissue (eWAT) belongs to visceral fat, mostly taken from the testes and epididymis in the abdominal cavity. When sampling, carefully remove other tissue and impurities that adhere. Brown adipose tissue (BAT) is mostly taken from the subcutaneous interscapular region and covered by iWAT. Care should be taken to cut away the iWAT and the tissue membranes in the BAT. Inguinal white adipose tissue (iWAT) is subcutaneous adipose tissue, mostly taken from the inguinal region. Carefully remove the lymph nodes from the iWAT and surrounding membranes. Purifying adipose tissue can avoid the interference of irrelevant cells. The adipose tissue should be soaked in Hanks buffer (containing $Ca^{2+}$ and $Mg^{2+}$) or RPMI 1640 whenever possible during the pretreatment and should be handled as gently and quickly as possible.
4) Please put in the amount of tissue as per recommended weight to avoid excessive tissue residues and insufficient digestion.
5) Discarding the supernate centrifuged by using a 50 mL centrifuge tube will cause the loss of some cells. Use a 15 mL centrifuge tube instead to continue the centrifugation, thus collecting more cells.
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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