# High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse)

## 1 Product Information

| Product Name | Model | Size |
|---|---|---|
| High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse) | DHTE-5001 | 50 T |

## 2 Description

The High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse) allows for the gentle, rapid and efficient dissociation of mouse implanted tumor tissues into single-cell suspensions. This optimized protocol allows for the highest possible number of single cell samples with high cell viability while preserving important surface antigenic epitopes of the cells. The single-cell suspension obtained can continue to be used for downstream experiments of tumor cells or TILs (tumor infiltrating lymphocytes) such as cell culture or cell sorting etc.

**Principle:** tumor tissues are dissociated into single-cell suspensions by a combination of mechanical dissociation and enzymatic digestion of the extracellular matrix (to maintain structural integrity of the tissue). RWD single cell suspension dissociator mainly plays the role of mechanical dissociation, while the High Activity Tumor Tissue Enzymatic Digestion Kit (Mouse) mainly digests the tissue by enzymatic digestion. After dissociation, the sample is filtered with a cell filter to remove tissue residues in the sample to obtain a single-cell suspension, and the cells obtained can be used immediately for subsequent experiments, such as primary cell culture, cell sorting, and single-cell sequencing etc.

## 3 Components

5 vials of reagents, including:

1 vial of enzyme A reagent (lyophilized powder);
1 vial of enzyme B reagent (lyophilized powder);
1 vial of enzyme C reagent (lyophilized powder);
1 vial of 8 mL Buffer B;
1 vial of 3 mL Buffer C.

## 4 Capacity

50 tumor tissue digestions, with 0.01 ~ 1.0 g of tumor tissue digested each time.

## 5 Transportation and storage

Transport in ice packs at 2 to 8°C;

The kits are stored separately by components. Enzyme C reagent is stored at -25 ~ -15°C, and the rest components are stored at 2 ~ 8°C, with validity period of 12 months.

## 6 Requirements for reagents and instruments

RPMI 1640 or DMEM;
(Optional) red blood cell lysis buffer (Solarbio: #R1010);
70 μm cell filter;
Constant temperature oscillator;
DSC-400 Single cell suspension dissociator (RWD);
Tissue processing tube* (RWD).

## 7 Method for use

### 7.1 Reagent preparation

1) **Prepare enzyme A solution:** dissolve and mix well each vial of enzyme A lyophilized powder by adding 2.75 mL of RPMI 1640 or DMEM, followed by frozen storage at -25 ~ -15℃ after aliquoting to avoid repeated freezing and thawing. The dissolved enzyme A solution remains stable for 6 months. If cell culture is carried out after tissue dissociation, sterile filtration (e.g., filtrate with a 0.22 μm needle filter) should be performed before aliquoting and storage.
2) **Prepare enzyme B solution:** dissolve and mix well enzyme B lyophilized powder with 6.875 mL of Buffer B, followed by frozen storage at -25 ~ -15℃ after aliquoting to avoid repeated freezing and thawing. The dissolved enzyme B solution remains stable for 6 months. If cell culture is carried out after tissue dissociation, sterile filtration (e.g., filtrate with a 0.22 μm needle filter) should be performed before aliquoting and storage.
3) **Prepare enzyme C solution:** dissolve the lyophilized powder in the enzyme C vial with 1.375 mL of Buffer C reagent, followed by frozen storage at -25 ~ -15℃ after aliquoting to avoid repeated freezing and thawing. The dissolved enzyme C solution remains stable for 6 months. If cell culture is carried out after tissue dissociation, sterile filtration (e.g., filtrate with a 0.22 μm needle filter) should be performed before aliquoting and storage.

### 7.2 Tumor tissue gentle enzymatic digestion

#### 7.2.1 DSC-400 Single cell suspension dissociator with HJ-400 heater is used

1) Add 2.3 mL of RPMI 1640 or DMEM, 50 μL of enzyme A, 125 μL of enzyme B, and 25 μL of enzyme C into the tissue processing tube, to prepare a mixed enzyme solution;
2) Remove the tumor mass from the mouse and cut it into small pieces of 2 ~ 4 mm in size, store the tissue mass temporarily with RPMI 1640 or DMEM, and weigh the target mass of the tissue mass with an electronic balance;
3) Transfer the tumor tissue to the tissue processing tube containing a mixed enzyme solution;
4) Tighten the tissue processing tube, invert it, and mount it into the channel of the DSC-400 single cell suspension dissociator, and install the heater (Note: make sure the sample is located in the rotor/stator area);
5) For soft tumor tissue, such as exonerating melanoma (induced by B16 cell line) or colon tumor (induced by CT26.wt cell line), run program M_Tumor_Heater_1; for hard tumor tissue, such as ionized breast tumor (induced by the 4T1 cell line) or moderately hard lung tumor (induced by the LLC cell line), run the program M_Tumor_Heater_2;
6) (Optional) for some hard tissues, some larger tissue blocks may still remain after running the program **M_Tumor_Heater_2**. In order to increase the cell yield, all the supernatant in the tissue processing tube was transferred to a new 15 mL centrifuge tube, leaving only the undigested tissue mass. Add 4 mL of RPMI 1640 or DMEM medium to the tissue processing tube containing the remaining tissue masses, and invert the tube, and mount it into the channel of the DSC-400 single cell suspension dissociator, without heater, run program **Mouse_Tumor_3,** and combine the cell suspension in the tissue processing tube with the supernatant in the 15 mL centrifuge tube;
7) After the program ends, proceed with step 11) in **7.2.2** until the operation finishes.

#### 7.2.2 DSC-400 Single cell suspension dissociator without HJ-400 heater is used

1) Add 2.3 mL of RPMI 1640 or DMEM, 50 μL of enzyme A, 125 μL of enzyme B, and 25 μL of enzyme C into the tissue processing tube, to prepare a mixed enzyme solution;
2) Remove the tumor mass from the mouse and cut it into small pieces of 2 ~ 4 mm in size, store the tissue mass temporarily with RPMI 1640 or DMEM, and weigh the target mass of the tissue mass with an electronic balance;
3) Transfer the tumor tissue to the tissue processing tube containing a mixed enzyme solution;
4) Tighten the tissue processing tube, invert and mount it into the channel of the DSC-400 single cell suspension dissociator (Note: make sure the sample is located in the rotor/stator area);
5) Run the program **Mouse_Tumor_1**;
6) After the program running is completed, remove the tissue processing tube from the DSC-400 single cell suspension dissociator;
7) Put the tissue processing tube in the constant temperature oscillating water bath kettle, rotate continuously at 150 rpm, and incubate for 40 minutes at 37°C. (Note: make sure the tissue processing tube is inverted to avoid the waste of tissues);
8) After incubation, invert the tissue processing tube and mount it into the channel of the DSC-400 single cell suspension dissociator.(Note: make sure the sample is located in the rotor/stator area);
9) Run the program **Mouse_Tumor_2**;
   ⚠ **Note: for soft tumor tissues, such as dissociated melanoma (induced by B16 cell line) or colon tumor (induced by CT26.WT cell line), run the program Mouse_Tumor_2 once; for hard tumor tissues, such as dissociated breast tumor (induced by 4T1 cell line) or moderately hard lung tumor tissue (induced by LLC cell line), run the program Mouse_Tumor_2 twice;**
10) (Optional) when working with tough tumors, after run the program **Mouse_Tumor_2** twice, some larger pieces of tissues may remain. To further increase the cell yield, allow the remaining tissue to settle and transfer all the supernatant to a new 15 mL centrifuge tube. Add 4 mL RPMI 1640 or DMEM to the tissue processing tube with the remaining tissue pieces. Mount it into the channel of the DSC-400 single cell suspension dissociator. Run program **Mouse_Tumor_3**. Combine the resulting cell suspension with the previously removed supernatant in 15 mL centrifuge tube;
11) Wet the 70 μm cell filter with 1 mL RPMI 1640 or DMEM medium, filter the cell sample with the wetted 70 μm cell filter and collect the cell suspension in a 50 mL centrifuge tube;
12) Rinse the tissue processing tube with 10 mL of RPMI 1640 or DMEM and filter with a 70 μm filter, and collect in a 50 mL centrifuge tube as described in Step 11;
13) Centrifuge the cell suspension at 500×g for 5 min and discard the supernatant completely;
14) Resuspend the cells with RPMI 1640 or DMEM to the required volume for subsequent experiments;
15) (Optional) To remove red blood cells, resuspend the cells resuspended in step 14) with 2 mL of erythrocyte lysis solution (Solarbio: #R1010), then incubate on ice for 3 ~ 5 min, followed by resuspension with 6 mL of RPMI 1640 or DMEM or PBS, centrifuge the cell suspension at 500×g for 5 min, and discard the supernatant completely.

## 8 Precautions

1) This kit is valid for 12 months, and RWD does not guarantee the validity when it is expired.
2) If downstream cell culture is carried out after tissue dissociation, all operations should be under aseptic conditions.
3) Approximately 2.5 mL of mixed enzyme solution is required for enzymatic digestion of 0.01 ~ 1.0 g of tumor tissue.
4) To analyze tumor infiltrating lymphocyte (TIL), it is recommended to reduce the amount of enzyme B in the enzyme mixture to 20% (e.g. add 25 μL of enzyme B to reduce the amount of enzyme B), which helps to better protect the cell surface epitopes but may have slight effect on cell yield and cell viability.
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 kit has been tested for transportation.

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



**RWD Life Science Co., Ltd.**
Web: www.rwdstco.com
Add: 850 New Burton Road, Suite 201, Dover, DE 19904, Kent, Delaware, USA
Add: 19F, Building 9A, Vanke Cloud City III, Liuxin 4 Street, Nanshan District, Shenzhen518000, Guangdong, P.R. China
Tel: +001-858-900-6602 +86-755-86111286
After-sales Service: +86-755-86111281
After-sales E-mail: service@rwdstco.com

EC REP Share Info GmbH
Heerdter Lohweg 83, 40549 Düsseldorf, Germany