High-Activity Tissue Enzyme Digestion Kit for General Use (Rat, Mouse)

Cat. NO. DHGT-5004

1. Description

The High-Activity Tissue Enzymatic Digestion Kit for General Use (the “Kit”) has been developed for gentle, rapid and efficient preparation of single cell suspensions from the spleen, lung, liver, kidney, and heart tissue (non-myocardial cells) of adult mice (6-8 Weeks) and from the heart tissue (myocardial cells) of neonatal rat and mouse within P1-P3 days. This optimized protocol can be used to obtain as many single cell samples as possible with high cell viability, while preserving the important cell surface epitopes. Such single cell suspensions attained can be used for downstream applications, including cell sorting and primary cell culture.

Main rationales: Single cell suspensions are prepared from adult or neonatal rat and mouse tissues by a combination of mechanical shearing and enzymatic digestion of extracellular matrix (ECM) (maintenance of tissue structure integrity). RWD Single Cell Suspension Dissociator mainly plays a role in mechanical dissociation, while the kit mainly digests the tissue by enzymatic dissociation. After dissociation, samples are filtered with cell filters to remove the tissue residues in them, so as to attain single cell suspensions of which the cells can be immediately used for subsequent experiments, including primary cell culture, cell sorting, and single cell sequencing.

1-1. Product Components

2 bottle of reagent in total, including

- 1 bottle: 2.1 ml of Enzyme A reagent (solution)
- 1 bottle: 1.4 ml of Enzyme B reagent (solution)

1-2. Storage

The Kit is dispensed and stored as components, of which the Enzyme A and Enzyme B solution are stored in a -20℃ freezer, with a shelf life of 6 months.

1-3. Test Capacity

The Kit has slight difference in tissues to be dissociated according to different tissue types to be processed. Please refer to Table 1. for details.

〈Table 1.〉

Tissue Type	Test Capacity	Initial Sample Size
Spleen of adult mouse	50 T	40~200 mg of spleen tissue to be processed per time
Lungs of adult mouse	50 T	100~300 mg of lung tissue to be processed per time
Liver of adult mouse	17 T	500~1200 mg of liver tissue to be processed per time
Kidneys of adult mouse	25 T	200~500 mg of kidney tissue to be processed per time
Heart of adult mouse	37 T	100~500 mg of heart tissue to be processed per time
Heart of neonatal rat and mouse	37 T	50~300 mg of heart tissue to be processed per time

1-4. Reagents and Apparatus Requirements

- DMEM or RPMI 1640 Media (Serum free)
- PBS
- Cell strainers (100 μm, 70 μm, 40 μm)
- Constant temperature shaking water bath
- Single Cell Suspension Dissociator (RWD #DSC-400)
- Single cell tube (RWD #SCT-25 or #SCT-100)
- (Optional) Red Blood Cell Lysis Buffer
- (Optional) High-efficiency Debris Removal Kit (RWD #DHDR-5006)

1-5. Precautions

- The Kit has 6-month shelf life, and RWD provides no warranty for the effectiveness of expired products.
- For any downstream cell culture to be performed subsequent to tissue dissociation, it is necessary to ensure that all steps are performed under sterile conditions.
- Enzyme reagents should be stored in different bottles and avoid repeated freezing and thawing. It should be used after dissolving on ice or in a freezer 4℃ to maintain its activity.

2. Protocol

2-1. Reagent Dispensing

- Prepare an appropriate amount of 1.5 ml tubes, divide Enzyme A and Enzyme B equally.
- Avoid repeated freezing and thawing during use.
- Store at -20℃.
- For cell culture experiments subsequent to tissue dissociation, Enzyme A and Enzyme B should be subject to sterile filtration before application.

2-2. Protocol for gentle tissue dissociation using DSC-400 Single Cell Suspension Dissociator (w/o Heat jacket)

1) Since the tissue types to be processed are different, corresponding enzyme mixture should be prepared in the tissue processing tubes (Single cell tube); refer to Table 2. for specific preparation:

〈Table 2.〉

Tissue Type	DMEM / RPMI 1640	Enzyme A	Enzyme B
Spleen of adult mouse	2.438 ml	37.5 μl	25 μl
Lungs of adult mouse	2.45 ml	25 μl	25 μl
Liver of adult mouse	4.84 ml	110 μl	50 μl
Kidneys of adult mouse	4.875 ml	75 μl	50 μl
Heart of adult mouse	2.425 ml	50 μl	25 μl
Heart of neonatal rat and mouse	2.425 ml	50 μl	25 μl

2) Remove corresponding tissues from mice or newborn rats, cut off the excess tissues, cut such tissues into small pieces of 2~4 mm, store these pieces in RPMI 1640 or DMEM temporarily, and weigh tissue pieces with target mass with an electronic balance.

3) Transfer the appropriate tissue into a tissue processing tube containing the enzyme mixture.

4) Tightly close the tube and attach it upside down onto the sleeve of DSC-400 Single Cell Suspension Dissociator (Note: it has to be ensured that the sample material is located in the area of the rotor/stator).

5) Run the processing program without heating of corresponding tissue according to different tissue types to be processed; refer Table 3.

〈Table 3.〉

Tissue Type	Program Name	Times of Run
Spleen of adult mouse	Mouse_Spleen_1	1
Lungs of adult mouse	Mouse_Lung_1	2
Liver of adult mouse	Mouse_Liver_1	1
Kidneys of adult mouse	Mouse_Kidney_1	1
Heart of adult mouse	Mouse_Heart_1	1

* Note: no program without heating is available for the heart of neonatal rat and mouse, but only the program with heating

6) After termination of the program, detach the tissue processing tube from DSC-400 Single Cell Suspension Dissociator.

7) Place the tissue processing tube in a constant temperature shaking water bath to incubate for different time depending on the type of tissue processed. Refer Table 4. for details. Keep the tissue processing tube upside down all the time, to avoid waste caused by tissue on the tube wall.

〈Table 4.〉

Tissue Type	Shaking Speed	Incubation Time	Incubation Temperature
Spleen of adult mouse	50 rpm	15 min	37℃
Lungs of adult mouse	50 rpm	15 min	37℃
Liver of adult mouse	50 rpm	30 min	37℃
Kidneys of adult mouse	50 rpm	30 min	37℃
Heart of adult mouse	50 rpm	15 min	37℃

8) After incubation, attach the tissue processing tube onto the sleeve of DSC-400 Single Cell Suspension Dissociator (Note: it has to be ensured that the sample material is located in the area of the rotor/stator).

9) Re-run the processing program of corresponding tissue according the different tissue types to be processed; refer to Table 5. for specific program.

〈Table 5.〉

Tissue Type	Program Name	Times of Run
Spleen of adult mouse	Mouse_Spleen_2	1
Lungs of adult mouse	Mouse_Lung_2	1
Liver of adult mouse	Mouse_Liver_2	1
Kidneys of adult mouse	Mouse_Kidney_2	1
Heart of adult mouse	Mouse_Heart_2	1

10) Wet a 70-μm cell strainer (Note: 40-μm for kidney tissue) with 1 ml of RPMI 1640 or DMEM, filter the cell sample with the wetted 40-μm or 70-μm cell strainer, and collect the cell suspension into a 50 ml centrifuge tube.

11) Flush the tissue processing tube with 9 ml of RPMI 1640 or DMEM, filter with the 40-μm or 70-μm cell strainer, and collect the suspension into a 50 ml centrifuge tube in step 10 (15 ml centrifuge tube can be used for centrifugation of fewer cells).

12) Centrifuge the cell suspension and run corresponding centrifugation program according to different tissue types to be processed; refer to Table 6. for specific program; aspirate the supernatant completely.

〈Table 6.〉

Tissue Type	Centrifugation Speed	Centrifugation Time
Spleen of adult mouse	300 x g	10 min
Lungs of adult mouse	600 x g	6 min
Liver of adult mouse	300 x g	10 min
Kidneys of adult mouse	300 x g	5 min
Heart of adult mouse	600 x g	5 min

13) (Optional) Remove the erythrocytes (i.e. spleen, lungs, liver, heart, kidneys), or the debris (i.e. livers, heart (adult mouse)) from the single cell obtained according to different tissue types to be processed.

To remove the debris, use RWD High-efficiency Debris Removal Kit (RWD #DHDR-5006);

a) For liver processing, re-suspend the cell sedimentation obtained in Step 12 with 6200 μl of cold PBS and mix well (not shaking), transfer to a 15 ml centrifuge tube, and add 1800 μl of cold debris removal reagent.

b) For heart processing, re-suspend the cell sedimentation obtained in Step 12 with 3100 μl of cold PBS and mix well (not shaking), transfer to a 15 ml centrifuge tube, and add 900 μl of cold debris removal reagent.

c) Gently blow with a 1 ml pipette for 10 times and mix well, then slowly drip 4 ml of cold PBS along the wall of the 15 ml centrifuge tube, centrifuge the cell suspension at 3000 x g, at 4℃, with the rinsing and descending speed of 5 and 3 respectively for 10 min; after centrifugation, the solution becomes stratified into 3 layers; thoroughly discard the uppermost two layers, collect the lower layer cells, add cold PBS solution to 10 ml, slightly reverse upside down for 3 times (not shaking for re-suspension), centrifuge the cell suspension at 1000 x g for 10 min, and aspirate supernatant completely.

(Optional) Use 2 ml of Red Blood Cell Lysis buffer to re-suspend cells processed in Step 13, place on ice and incubate for 3~5 min, stop reaction with 9 ml of RPMI 1640 or DMEM, centrifuge the cell suspension at 500 x g for 5 min, and aspirate supernatant completely.

14) Re-suspend the cells with RPMI 1640 or DMEM to the required volume for further experimental applications.

2-3. Protocol for gentle tissue dissociation using DSC-400 Single Cell Suspension Dissociator (with heat jacket)

1) Since the tissue types to be processed are different, corresponding enzyme mixture should be prepared in the tissue processing tubes (Single cell tube); refer to Table 2. for specific preparation:

〈Table 2.〉

Tissue Type	DMEM / RPMI 1640	Enzyme A	Enzyme B
Spleen of adult mouse	2.438 ml	37.5 µl	25 µl
Lungs of adult mouse	2.45 ml	25 µl	25 µl
Liver of adult mouse	4.84 ml	110 µl	50 µl
Kidneys of adult mouse	4.875 ml	75 µl	50 µl
Heart of adult mouse	2.425 ml	50 µl	25 µl
Heart of neonatal rat and mouse	2.425 ml	50 µl	25 µl

2) Remove corresponding tissues from mice or newborn rats, cut off the excess tissues, cut such tissues into small pieces of 2~4 mm, store these pieces in RPMI 1640 or DMEM temporarily, and weigh tissue pieces with target mass with an electronic balance.

3) Transfer the appropriate tissue into a tissue processing tube containing the enzyme mixture.

4) Tightly close the tube and attach it upside down onto the sleeve of DSC-400 Single Cell Suspension Dissociator (Note: it has to be ensured that the sample material is located in the area of the rotor/stator).

5) Run the processing program with heating of corresponding tissue according to different tissue types to be processed; refer Table 7.

〈Table 7.〉

Tissue Type	Program Name	Times of Run
Spleen of adult mouse	M_Spleen_Heater_1	1
Lungs of adult mouse	M_Lung_Heater_1	1
Liver of adult mouse	M_Liver_Heater_1	1
Kidneys of adult mouse	M_Kidney_Heater_1	1
Heart of adult mouse	M_AHeart_Heater_1	1
Heart of neonatal rat and mouse	M_NeoHeart_Heater_1	1

6) After termination of the program, detach the tissue processing tube from DSC-400 Single Cell Suspension Dissociator.

7) Wet a 70-µm cell strainer (Note: 40-µm for kidney tissue) with 1 ml of RPMI 1640 or DMEM, filter the cell sample with the wetted 40-µm or 70-µm cell strainer, and collect the cell suspension

into a 50 ml centrifuge tube.

8) Flush the tissue processing tube with 9 ml of RPMI 1640 or DMEM, filter with the 40-μm or 70-μm cell strainer, and collect the suspension into a 50 ml centrifuge tube in step 10 (15 ml centrifuge tube can be used for centrifugation of fewer cells).

9) Centrifuge the cell suspension and run corresponding centrifugation program according to different tissue types to be processed; refer to Table 6. for specific program; aspirate the supernatant completely.

〈Table 6.〉

Tissue Type	Centrifugation Speed	Centrifugation Time
Spleen of adult mouse	300 x g	10 min
Lungs of adult mouse	600 x g	6 min
Liver of adult mouse	300 x g	10 min
Kidneys of adult mouse	300 x g	5 min
Heart of adult mouse	600 x g	5 min

10) (Optional) Remove the erythrocytes (i.e. spleen, lungs, liver, heart, kidneys), or the debris (i.e. livers, heart (adult mouse)) from the single cell obtained according to different tissue types to be processed.

To remove the debris, use RWD High-efficiency Debris Removal Kit (RWD #DHDR-5006);

a) For liver processing, re-suspend the cell sedimentation obtained in Step 12 with 6200 μl of cold PBS and mix well (not shaking), transfer to a 15 ml centrifuge tube, and add 1800 μl of cold debris removal reagent.

b) For heart processing, re-suspend the cell sedimentation obtained in Step 12 with 3100 μl of cold PBS and mix well (not shaking), transfer to a 15 ml centrifuge tube, and add 900 μl of cold debris removal reagent.

c) Gently blow with a 1 ml pipette for 10 times and mix well, then slowly drip 4 ml of cold PBS along the wall of the 15 ml centrifuge tube, centrifuge the cell suspension at 3000 x g, at 4℃, with the rinsing and descending speed of 5 and 3 respectively for 10 min; after centrifugation, the solution becomes stratified into 3 layers; thoroughly discard the uppermost two layers, collect the lower layer cells, add cold PBS solution to 10 ml, slightly reverse upside down for 3 times (not shaking for re-suspension), centrifuge the cell suspension at 1000 x g for 10 min, and aspirate supernatant completely.

(Optional) Use 2 ml of Red Blood Cell Lysis buffer to re-suspend cells processed in Step 13, place on ice and incubate for 3~5 min, stop reaction with 9 ml of RPMI 1640 or DMEM, centrifuge the cell suspension at 500 x g for 5 min, and aspirate supernatant completely.

11) Re-suspend the cells with RPMI 1640 or DMEM to the required volume for further experimental applications.