High Activity General Tissue Enzymatic Digestion Kit II Instructions

Product Information

Product Name	Model	Specification
High Activity General Tissue Enzymatic Digestion Kit II	DHGTP-5004	50 T (depending on tissue type)

Product Description

High Activity General Tissue Enzymatic Digestion Kit II (the "Kit") can prepare heart (non-cardiomyocytes), lung, spleen, liver, kidney, lymph node, testis and thymus tissue of adult mice into single cell suspension gently, quickly and efficiently. This optimized protocol 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 be applied in downstream experiments such as primary cell culture, cell sorting and single cell sequencing.

Main principle: Mechanical dissociation is in combination with enzymatic digestion of the extracellular matrix (while maintaining the intactness of tissue structure) to prepare the tissue into single cell suspension. RWD Single Cell Suspension Dissociator is chiefly used for mechanical dissociation, while the Kit mainly digests the tissue through enzymatic digestion. After dissociation, the cell suspension is filtered through the cell strainers to remove tissue residues to obtain single cell suspension.

Product Components

Product Name	Components	Quantity	Storage
High Activity General Tissue Enzymatic Digestion Kit II	Enzyme A Reagent (powder)	1 vial	2 ~ 8℃
	Enzyme B Reagent (powder)	1 vial	2 ~ 8℃
	Enzyme C Reagent (powder)	1 vial	-25 ~ -15℃
	Buffer B (solution)	1 vial	2 ~ 8℃
	Buffer C (solution)	1 vial	2 ~ 8℃

Test Capacity

Recommendation for single process of tissue:

Tissue Type	Capacity	Initial Sample Dosage
Adult Mouse Heart	50 T	100 ~ 500 mg to be processed per time
Adult Mouse Liver	34 T	500 ~ 850 mg to be processed per time
Adult Mouse Spleen	70 T	40 ~ 400 mg to be processed per time
Adult Mouse Lung	34 T	100 ~ 300 mg to be processed per time
Adult Mouse Kidney	34 T	200 ~ 500 mg to be processed per time
Adult Mouse Lymph Node	100 T	20 ~ 400 mg to be processed per time
Adult Mouse Testis	100 T	50 ~ 600 mg to be processed per time
Adult Mouse Thymus	100 T	20 ~ 600 mg to be processed per time

Storage & Transportation

- ✧ The Kit should be transported at 2 ~ 8℃.
- ✧ The Kit is separated into two packages due to different storage temperatures, please store them separately according to the attached temperature label.
- ✧ It is recommended that all the enzyme reagents should be dissolved separately, mixed evenly and stored in small packages. Avoid repeated freezing, thawing and vigorous shaking.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Reagents & Instruments

Reagents	PBS	RPMI 1640 or DMEM
	Red Blood Cell Lysis Buffer	HBSS (with Ca^{2+} and Mg^{2+})
	High Efficiency Debris Removal Kit (Optional, RWD: # DHDR-5006)	
Consumables	Tissue Processing Tube (RWD)	Heater (RWD: # HJ-400)
	40 μm / 70 μm Cell Strainers	0.22 μm Syringe Filter (Optional)
Instruments	Single Cell Suspension Dissociator (RWD)	High-Speed Benchtop Refrigerated Centrifuge (RWD: # M1416R)

Operation

Preparation

(1) Preparation of enzyme A solution: Dissolve the enzyme A reagent (powder) with 10.5 mL of HBSS (with $Ca^{2+}$and Mg^{2+}), subpackage the solution and store at -25 ~ -15℃. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃ and it should avoid repeated freezing, thawing and vigorous shaking.

(2) Preparation of enzyme B solution: Dissolve the enzyme B reagent (powder) with 2.6 mL of Buffer B, subpackage the solution and store at -25 ~ -15℃. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃ and it should avoid repeated freezing, thawing and vigorous shaking.

(3) Preparation of enzyme C solution: Dissolve the enzyme C reagent (powder) with 2.6 mL of Buffer C, subpackage the solution and store at -25 ~ -15℃. The enzyme solution can be stored stably for 6 months at -25 ~ -15℃ and it should avoid repeated freezing, thawing and vigorous shaking.

Mechanized Protocol

(1) Depending on the tissue type, prepare the enzyme mixture in the tissue processing tube. If cell culture is required, aseptically filter the enzyme mixture through a 0.22 μm syringe filter and ensure that after the filtration, the total volume of the enzyme mixture is 2.5 mL or 5 mL. The subsequent operations should be completed on the aseptic operating platform. For detailed proportions of preparation, refer to Table 1.

Table 1 Preparation proportions for different tissues

Tissue Type	RPMI 1640 / DMEM	Enzyme A	Enzyme B	Enzyme C
Adult Mouse Heart	2.225 mL	200 μL	50 μL	25 μL
Adult Mouse Liver	2.125 mL	300 μL	50 μL	25 μL
Adult Mouse Spleen	2.315 mL	150 μL	10 μL	25 μL
Adult Mouse Lung	2.150 mL	300 μL	25 μL	25 μL
Adult Mouse Kidney	4.620 mL	300 μL	30 μL	50 μL
Adult Mouse Lymph Node, Testis, Thymus	2.365 mL	100 μL	10 μL	25 μL

(2) Isolate target tissues from mice and remove excess adipose tissue, surrounding membranes, and residual vasculature. Mince the tissues into 2 ~ 4 mm of fragments and place the tissue fragments in the 4°C RPMI 1640 or DMEM for short-term preservation.

⚠ Note: Since the single cell suspension from digested kidney tissue may contain aggregates (e.g. kidney tubules or glomeruli), it is recommended to further mince the tissue fragments with scissors and wash the fragments with medium. Transfer tissue fragments to a 100 μm cell strainer and aspirate 2 mL of medium per rinse (repeat 2-3 times) to wash the fragments.

(3) Weigh target weight of tissue fragments according to the ***Test Capacity*** and transfer the fragments to the

tissue processing tube containing enzyme mixture.

(4) Tighten the tissue processing tube, invert and mount it in the bushing of the single cell suspension dissociator with the heater.

⚠ Note: Ensure the sample is in the area where the rotor/stator is located.

(5) Depending on the tissue type, run the appropriate program. For details, refer to Table 2.

Table 2 Programs for different tissues

Tissue Type	Program Name
Adult Mouse Heart	M_AHeart_Heater_1
Adult Mouse Liver	M_Liver_Heater_1
Adult Mouse Spleen, Lymph Node, Thymus	M_Spleen_Heater_1
Adult Mouse Lung	M_Lung_Heater_1
Adult Mouse Kidney, Testis	M_Kidney_Heater_1

(6) After the program is finished, remove the tissue processing tube from the single cell suspension dissociator.

⚠ Note: For removing aggregates (e.g., kidney tubules or glomeruli) of kidney tissue, after program completion, briefly centrifuge the tissue processing tube (100×g, 5 s), aspirate the supernatant and filter the supernatant through the 40 µm cell strainer. This method can also improves filtration efficiency when encountering slow flow rates.

(7) Wet a 70 µm cell strainer with 1 mL of RPMI 1640 or DMEM (for kidney tissue, use a 40 µm cell strainer), filter the cell suspension through the wetted cell strainer and collect it to a 50 mL centrifuge tube.

(8) Rinse the tissue processing tube with 10 mL of RPMI 1640 or DMEM, filter the suspension through the 70 µm cell strainer and collect it to the 50 mL centrifuge tube mentioned in step (7).

⚠ Note: For kidney tissue, rinse the cell strainer with 10 mL of RPMI 1640 or DMEM and collect the solution in the 50 mL centrifuge tube, avoiding re-rinsing the tissue processing tube.

⚠ Note: For low cell yields obtained from heart tissue, transfer the suspension to a 15 mL centrifuge tube for centrifugation.

(9) Centrifuge the cell suspension by running the appropriate program. After centrifugation, please discard the supernatant carefully to avoid aspirating the cell pellet.

Table 3 Centrifuge programs for different tissues

Tissue Type	Centrifuge Speed	Centrifuge Time
Adult Mouse Heart	600×g	5 min
Adult Mouse Liver	300×g	10 min
Adult Mouse Spleen, Lymph Node, Thymus, Testis	500×g	5 min
Adult Mouse Lung	600×g	6 min
Adult Mouse Kidney	300×g	5 min

(10) Depending on the tissue type, perform debris removal for the obtained single cell suspension (e.g., heart, liver), or the red blood cell removal (e.g., heart, liver, spleen, lung, kidney). For lymph node, testis and thymus tissues, the red blood cell lysis is not required.

⚠ Note: When the cell debris removal and the red blood cells removal are both needed, it is recommended to perform the removal of cell debris first.

(Optional)To remove the cell debris, use the High Efficiency Debris Removal Kit (RWD: #DHDR-5006).

(a) **For liver**: Resuspend the cell pellet (do not shake) obtained in step (9) with 6200 µL of cold PBS and transfer the suspension to a 15 mL centrifuge tube. Add 1800 µL of cold debris removal reagent to the centrifuge tube and perform step (c).

(b) **For heart**: Resuspend the cell pellet (do not shake) obtained in step (9) with 3100 µL of cold PBS and transfer the suspension to a 15 mL centrifuge tube. Add 900 µL of cold debris removal reagent to the centrifuge tube and perform step (c).

(c) Gently blow the suspension 10 times by an appropriate pipette and slowly add 4 mL of cold PBS to the 15 mL centrifuge tube along the tube wall. Perform gradient centrifugation at 3000×g, 4°C for 10 min with acceleration 9 and deceleration 3. After centrifugation, the suspension will separate into four distinct layers. Aspirate and discard the upper three layers while retaining the bottom cell pellet.

(Optional) Depending on the tissue type, perform the appropriate method of red blood cell lysis to resuspend the cell pellet and remove the red blood cells.

Table 4 Red blood cell lysis methods for different tissues

Tissue Type	Tissue Volume	RBC Lysis Buffer Volume	Recommended Lysis Time	Best Lysis Temperature
Adult Mouse Heart	Approximately 160 mg	1 mL	2 min	2 ~ 8°C
Adult Mouse Liver	Approximately 850 mg	2 mL	3 min	2 ~ 8°C
Adult Mouse Spleen	Approximately 130 mg	3 mL	5 min	2 ~ 8°C
Adult Mouse Lung	Approximately 200 mg	2 mL	4 min	2 ~ 8°C
Adult Mouse Kidney	Approximately 300 mg	2 mL	4 min	2 ~ 8°C

After lysis, add 10 mL of medium to terminate the resuspension. Refer to Table 3, centrifuge the cell suspension by appropriate program and completely discard the supernatant after centrifugation.

⚠ Note: The volume of red blood cell lysis buffer and lysis time may be adjusted based on the experimental conditions. If the pellet remains distinctly red after the lysis, it is suggested to perform the lysis again with reduced buffer volume and shorter lysis time.

(11) Resuspend the cell suspension with RPMI 1640 or DMEM or other buffers to required volume for subsequent experiments.

⚠ Note: For the floc in the cell suspension, it is suggested to filter the suspension through the 40 µm cell strainer.

Precautions

(1) The Kit is valid for 12 months and RWD shall not guarantee the validity of expired products.

(2) 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.

(3) The enzyme reagent should be stored in small packages, and avoid repeated freezing and thawing. It should be used after dissolving on ice or in a refrigerator at 4°C to maintain its activity.

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

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