Tissue Single-Cell Preparation Kit Instruction Manual

Cat#：5080421、5080422

【Product Description】

The Biosci Tissue Single-Cell Preparation Kit enables gentle and rapid isolation of cells from fresh solid tissues while maintaining high cell viability, minimal cell debris/clumping, and intact cell surface antigen epitopes. Validated across various human and mouse tissues, the kit provides high-quality single-cell suspensions suitable for downstream applications such as flow cytometry, magnetic bead sorting, cell culture, and single-cell sequencing.

[Product Specifications]

Product Name	Product Cat#	Package	Component Cat#	Component Name	Component Specification	Qty
Tissue Single-Cell Preparation Kit	5080421	4 RCTs	5080421-1	Lyophilized Enzyme Reagent (Enzyme A)	2 mg/vial	1 vial
				Lyophilized Enzyme Reagent (Enzyme B)	20 mg/vial	1 vial
				Lyophilized Enzyme Reagent (Enzyme C)	1 mg/vial	1 vial
			5080421-2	Tissue Single-Cell Preparation Buffer	20 mL/bottle	1 bottle
			5080421-3	Tissue single-cell preparation tube	/	4 units
	5080422	24 RCTs	5080422-1	Lyophilized Enzyme Reagent (Enzyme A)	2 mg/vial	6 tubes
				Lyophilized Enzyme Reagent (Enzyme B)	20 mg/vial	6 tubes
				Lyophilized Enzyme Reagent (Enzyme C)	1 mg/vial	6 tubes
			5080422-2	Tissue Single-Cell Preparation Buffer	20 mL/bottle	6 bottles

*5080422 must be used with the tissue single-cell preparation tube (Catalog No.: 5080432). Please prepare separately.

[Product Composition]

Consists of lyophilized enzyme powder and buffer solution.

[Product Principle]

The tissue single-cell preparation kit contains a mixture of digestive enzymes with different mechanisms of action. It rapidly dissociates tissue into a single-cell suspension while maintaining cell viability by disrupting interstitial components and intercellular connections through mechanical grinding/shearing and enzymatic digestion.

[Storage Conditions and Shelf Life]

- The lyophilized enzyme reagent should be stored at -25 ℃ to -15 ℃; the tissue single-cell preparation buffer should be stored at 2 ℃ to 8 ℃; the tissue single-cell preparation tubes should be stored at room temperature, 2 ℃ to 30 ℃.
- The kit has a shelf life of 1 year.

[Instructions for Use]

1. Materials to Prepare

Reagents	Consumables	Equipment
Fluorescent cell analysis dye (AO/PI)	Pasteur pipette (5 mL)	Fluorescence inverted microscope
1x PBS buffer	Sterile ophthalmic scissors/forceps	High-speed refrigerated centrifuge
1HBSS buffer solution	40 μm/70 μm/100 μm sterile filters	Fluorescent cell counter
RBC lysis buffer	1.5 mL low-adsorption centrifuge tubes	Adjustable pipettes
0.4% Trypan blue solution	15 mL/50 mL low-adsorption centrifuge tubes	Hemocytometer
	6 cm sterile culture dishes	Biosci Tissue Dissociator

2. Operating Procedure

➢ Preparation before the experiment:

a. Bring the Biosci tissue single-cell preparation buffer to room temperature for standby.

b. Place PBS and HBSS on ice to pre-cool for later use.

c. Sterilize ophthalmic scissors, ophthalmic forceps, and other dissociation-related instruments for later use.

d. Aliquot the trypan blue solution and fluorescent dye solution used for cell counting for later use.

➢ Reagent Preparation:

a. Preparation of Lyophilized Enzyme Reagent (Enzyme A): Add 2 mL of Tissue Single-Cell Preparation Buffer reagent to each vial of lyophilized enzyme reagent (Enzyme A), and mix thoroughly by pipetting to ensure complete dissolution.

b. Preparation of Lyophilized Enzyme Reagent (Enzyme B): Add 2 mL of Tissue Single-Cell Preparation Buffer reagent to each vial of lyophilized enzyme reagent (Enzyme B), and mix thoroughly by pipetting to ensure complete dissolution.

c. Preparation of Lyophilized Enzyme Reagent (Enzyme C): Add 2 mL of Tissue Single-Cell Preparation Buffer to each vial of Lyophilized Enzyme Reagent (Enzyme C), and mix thoroughly by pipetting to ensure complete dissolution.

d. Preparation of Mixed Enzyme Solution: Combine 2 mL of Enzyme A solution, 2 mL of Enzyme B solution, 2 mL of Enzyme C solution, and 14 mL of Tissue Single-Cell Preparation Buffer in a 50 mL centrifuge tube, and mix thoroughly to prepare a total of 20 mL of Tissue Single-Cell Preparation Solution. This can be evenly divided into 4 portions to complete the dissociation of 4 tissue samples.

The range of tissue weight and the recommended volume of the tissue single-cell preparation solution can be found in the table below:

Range of Tissue Weight	Recommended Volume of Tissue Single-Cell Preparation Solution
10 mg < Tissue Weight ≤ 50 mg	0.5 mL~1 mL
50 mg < Tissue Weight ≤ 100 mg	1 mL~2 mL
100 mg < Tissue Weight ≤ 500 mg	2 mL~3 mL
500 mg < Tissue Weight ≤ 1 g	3 mL~5 mL
Tissue Weight > 1 g	5 mL~7 mL

Note: After aliquoting, store frozen at -25 ℃ to -15 ℃**. Avoid repeated freeze-thaw cycles. This**

solution can be stored frozen for up to 1 year.

➢ Tissue Dissociation:

a. Wash & Weigh: Transfer fresh tissue to a culture dish containing HBSS buffer using sterile ophthalmic forceps. Wash 3× sequentially and blot dry to remove excess buffer..

b. Add Tissue: Pipette a small amount of the prepared or fully thawed tissue single-cell preparation solution into the tissue. Use sterile enzyme-free scissors to mince the tissue. Transfer the tissue to the tissue single-cell preparation tube and continue to add the tissue single-cell preparation solution according to the weight of tissue.

c. Tube Installation: Tighten the preparation tube and place it steadily into the corresponding channel of the Tissue Dissociator.

Figure 1: Tube Installation

d. Program Setup: The Biosci Tissue Dissociator is pre-programmed with 5 tissue dissociation protocols optimized for mouse tissues including kidney, liver, testis, heart, lung, spleen, thymus, brain, etc. Users can also customize parameters for each channel, including motor speed, forward rotation duration, reverse rotation duration, cycle count, and heating duration. Each channel can be independently configured with a unique set of parameters based on tissue type and characteristics.

The Biosci Tissue Dissociator is pre-programmed with 5 optimized protocols for the following tissue types:

Program	Statement	Remark
S1	Program 1	Suitable for tissues such as mouse testis, heart, kidney, brain, spleen, thymus, brain lymphoma, and melanoma.
S2	Program 2	Fast grind – for tissue ≤20 mg
S3	Program 3	High-speed grind - suitable for tissues such as mouse lung.
S4	Program 4	Before grinding, tissues need to be soaked in the tissue single-cell preparation solution.
		——Suitable for mouse intestinal tissues, lymph nodes, breast cancer, etc.
S5	Program 5	Large volume tissue (tissue weight > 700 mg)

e. Cell Microscopy: After the program is completed and the stage exits the chamber, remove the preparation tube and gently unscrew it. Mix 10 μL of the cell suspension with Trypan blue solution at a 1:1 volume ratio for staining, and observe tissue dissociation under a microscope. If the tissue is fully digested with no clumping, aggregates, or debris visible, the cell suspension is considered

qualified，and digestion can be stopped. If there is a significant amount of residual tissue and severe cell clumping, the cell suspension is considered unqualified, and digestion can be continued for an additional 5 to 10 minutes, then observe and assess whether the cell suspension meets the standards.

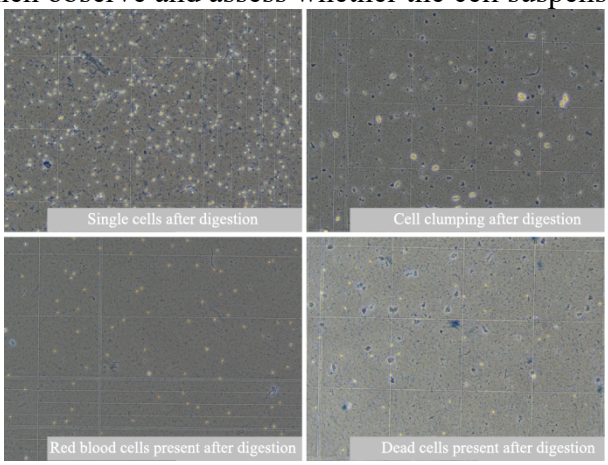

Figure 1: Single cells after digestion; Figure 2: Cell clumping after digestion; Figure 3: Red blood cells present after digestion; Figure 4: Dead cells present after digestion.

f. Cell Filtration: Filter the dissociated cell suspension through a disposable sterile filter (40 μm, 70 μm, or 100 μm) into a 50 mL low-adhesion centrifuge tube. Rinse the walls of the centrifuge tube 1-2 times with PBS to ensure complete transfer of cells, and adjust the volume of the filtered suspension to 15 mL to 25 mL with PBS.

g. Centrifugation and Resuspension: Centrifuge the cell suspension obtained from the previous step at 350 g for 5 minutes. After centrifugation, carefully remove the supernatant along the wall of the centrifuge tube with a Pasteur pipette until approximately 1 mL of supernatant remains, then resuspend the cell pellet in the remaining volume. The supernatant can be temporarily stored in the centrifuge tube.

h. Cell counting: Mix 10 μL of cell suspension with trypan blue solution at a 1:1 volume ratio for staining. Observe the cell status under a microscope, and calculate cell concentration and viability using a fluorescence counter or counting board.

[Precautions]

- To achieve optimal dissociation results, it is recommended to prepare this kit for immediate use to avoid repeated freezing and thawing.
- Reagents should be temporarily stored on ice, and the tissue single-cell preparation solution should be used promptly for subsequent experiments.
- If cell culture is performed after tissue dissociation, ensure that all operations are conducted under sterile conditions.
- The shelf life of this kit is 1 year; the effectiveness of expired products is not guaranteed.
- If the proportion of red blood cells is greater than 20%, red blood cell lysis is required first.
- If there are many cell impurities or cell debris, impurity removal is necessary.

[Explanation of Product Label Symbols]

Product Label Symbols	Explanation	Product Label Symbols	Explanation
REF	Product Number	LOT	Batch Code
	Production Date		Temperature Limit
	Shelf Life		See Instructions for Details
	Avoid Sunlight		Manufacturer

[Approval Date of Product Manual]

June 11, 2024

[Company Information]

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