SeekMate Tissue Dissociation Kit A Pro
User Manual

【Product Name】

SeekMate Tissue Dissociation Kit A Pro

【Model/Specification】

K01801-08, 8 tests;

K01801-30, 30 tests.

【Intended use】

This product is intended for tissue dissociation of human or mouse tissues in vitro. Currently, it is only used for scientific research and not for reinfusion therapy.

【Test Principle】

The connections between extracellular matrix and cells in tissues are composed of a series of different proteins and other biological components. Through specific combinations of proteases, these connections can be digested to achieve tissue dissociation.

【Main Components】

The kit consists of enzymes H, R, and A. Each enzyme component is present in a solution state, and the two types of tissue dissociation kits are suitable for different tissue samples. The specifications are as follows:

	Component	**8 tests**	**30 tests***
SeekMate Tissue Dissociation Kit A Pro	Enzyme H	290 µL	1080 µL
	Enzyme A	210 µL	780 µL
	Enzyme R	345 µL	1300 µL

***It is recommended to store the 30 tests packaging reagents in 2-3 separate batches after the first thawing to avoid repeated freeze-thaw cycles.**

*** For a single reaction, the required enzymes include: 30 µL of enzyme H, 36 µL of enzyme R, and 21.6 µL of enzyme A.**

【Storage Condition and Shelf Life】

The unopened reagent kit is stored at -20 ± 5 ℃ and has a validity period of 12 months.

【Sample Requirements】

1. Applicable sample type:

The reagent kit is suitable for human or mouse liver, lung tissue, mouse tumor tissue, and human tumor samples (not suitable for brain tumors).

2. Sample Collection Precautions:

2.1 Try to avoid collecting necrotic, calcified, sclerotic, or fibrotic areas during sampling, especially in lesion tissues.

2.2 If the surgical operation involves cutting tissue with an electric knife, remove necrotic tissue that has come into contact with the knife.

2.3 Ensure that the target tissue is not frozen during operation.

2.4 Following sampling, trim tissue to remove non-target parts as soon as possible and place it into a tissue preservation solution to avoid influencing the final sequencing results.

2.5 If the specimen is from an elderly animal, please send it to the laboratory as soon as possible for dissociation.

2.6 It is important to consider the experimental model in advance as it may have an impact on the cells. For example, if silica particles are introduced into the mouse lungs, and the density of silica is similar to that of cells, it may be difficult to completely remove the silica particles in subsequent steps.

2.7 It is important to consider in advance whether drug therapy will affect the cells. For example, after tumor treatment, the fragments of dead tumor cells are difficult to distinguish from normal cells, which can affect the accuracy of cell nucleation rate.

3. Sample storage

Collect suitable tissue sample and wash it off the blood using PBS or physiological saline. Place the washed tissue sample in a 1 mL or larger storage tube filled with tissue preservation solution. The storage period should not exceed 48 hours.

4. Sample transportation

Tissue samples stored in tissue preservation solution should be transported with the ice bag at 4℃.

【Tissue Dissociation Procedure】

1. Thaw a single tube of tissue-specific dissociation enzyme on ice.

   Prepare 50 ml of cell resuspension buffer on ice: 1640 medium + 2% FBS.

Prepare the dissociation solution as follows.

Type	Tissue Weight of 0-150 mg	Tissue Weight of 150-300 mg
Enzyme H	30 µL	50 µL
Enzyme R	36 µL	60 µL
Enzyme A	21.6 µL	36 µL
1640 medium + 2% FBS	2.92 mL	4.86 mL
Total Volume	3.00 mL	5.00 mL

2. Tissue preparation: add 1 × PBS solution to a 6-well plate, remove the tissue from the tissue preservation solution and place it in it for washing, wash off the residual blood and mucus. Take a proper amount of washed sample, add it to the dissociation solution, and cut it into 1 mm tissue blocks using surgical scissors.

   Note 1: Cut the tissue into bean-sized pieces in six-hole plate, wash off blood and mucus; puncture biopsy sample can be directly centrifuged into the storage tube without washing step to reduce washing loss. Tissue after drug treatment or fragile modeling can be washed with cell resuspension buffer.

   Note 2: Small tissues can be cut in a 1.5 mL centrifuge tube with 200 µL of dissociation reagent and then transfer it to a larger digestion tube.

3. Incubate the dissociation solution and tissue block in a hybridization oven or water bath at 37℃ for 15-40 minutes for optimal tissue dissociation. Every 10 minutes, mix the dissociation solution using a wide orifice pipette tip. If there are large tissue pieces, you can further mince them with scissors.

   Note: For fragile samples or new tissue types being tested, perform an AO/PI check every 10 minutes after mixing. Count and monitor the cells. If the tissue is not completely dissociated or there is a significant decrease in cell viability, stop the incubation. Adjust the concentration of the dissociation solution if necessary.

4. After complete dissociation, gently mix the dissociation solution and filter it through a sterile 40-70µm cell strainer.

   Note: Prior to using the cell strainer, rinse it with cell resuspension buffer. It is recommended to first use a 70 µm cell strainer at this step to minimize loss. For samples with viscous dissociation solution, dilute the dissociation solution with resuspension buffer before filtering.

5. Rinse the cell strainer with 5 ml of 1640 medium + 2% FBS.

6. Mix and centrifuge the filtered cell suspension at 4 ℃, 300 g, for 5 minutes. Then remove the supernatant.

   Note: If the cell count is less than 50,000, it is recommended to centrifuge at 400 g.

7. Resuspend the cell pellet in 1 mL of 1640 culture medium + 2% FBS. Mix well and perform AO/PI staining for cell counting and monitoring.

Note: The recommended resuspension concentration is 700-1200 cells/μL. Adjust the resuspension volume based on the expected concentration.

8. Red blood cell lysis: Add three times the volume of red blood cell lysis solution (e.g., Solarbio R1010) to the cell suspension. Invert and mix, then incubate at room temperature for 2-5 minutes. Stop the red blood cell lysis by adding an equal volume of 1640 culture medium + 2% FBS solution.

9. Invert and mix, then centrifuge at 4 ℃, 300 g, for 5 minutes. Then remove the supernatant.

   Note 1: If the cell count is less than 50,000, it is recommended to centrifuge at 400 g. If there are cell aggregates or impurities in step 7, it is advisable to filter the suspension through a 40 μm cell strainer before centrifugation.

   Note 2: For other brands of red blood cell lysis reagents, follow the manufacturer's SOP for usage instructions.

10. Resuspend the cell pellet in 1 ml of 1640 culture medium + 2% FBS. Mix well and perform AO/PI staining for cell counting and monitoring.

    Note: The recommended resuspension concentration is 700-1200 cells/μL. Adjust the resuspension volume based on the expected concentration.

11. If the cell assay is testing qualified, temporarily store the cells on ice or at 4 ° C and proceed with subsequent experiments. If there are significant cell fragments, optimization steps are required. After passing the qualification test, proceed with subsequent experiments.

    Quality control criteria (using single-cell sequencing experiment as an example): Cell viability > 90%; Cell aggregation rate < 10%; Nucleated cell rate (proportion of cells with a nucleus) > 70%; Concentration of viable cells: 700-1200 cells/μL; Cell diameter range: 5-40 μm.

【Judgment Value or Reference Interval】

This reagent kit is a sample preparation kit. The purified product obtained from sample processing can be used for further in vitro diagnostic tests, however, the reagent kit itself DOES NOT HAVE ANY POSITIVE JUDGMENT VALUES OR REFERENCE INTERVALS.

【Explanation and optimization of test results】

1. Interpretation of test results

The quality of the dissociated single cell suspension depends heavily on the sample quality and its own properties. Poor sample quality, inability to separate necrotic tissue, or high levels of endogenous proteases can all result in poor quality single-cell suspension with excessive fragments, which may lead to failure in subsequent experiments. When working with poor quality samples, it is recommended to select suitable parts for the experimental operation. In cases where there are excessive fragments, low nucleated cell rates or low viability, additional procedures can be conducted to eliminate fragments, dead cells, or filter the cells. Please refer to the attached table for recommended consumables.

2. Optimization strategies

2.1. Low viability (viability < 80%):

Recommend using flow cytometry or a Dead Cell Removal Kit (*Miltenyi Biotec) for cell sorting. Please ensure a minimum of 500,000 viable cells for subsequent experimental needs. This is commonly observed in necrotic tissues, injured tissue models, or tumor samples after drug treatment. For viability between 80-85%, resuspend and mix the cells with 4 mL of 1640 culture medium + 10% FBS. Centrifuge the cell suspension again and wash it once more.

2.2. Low nucleated cell rate (nucleated cell rate < 55%):

Recommend using flow cytometry or a Debris Removal Kit (*Miltenyi Biotec) for optimization. Please ensure a minimum of 500,000 viable cells for subsequent experimental needs. This is commonly observed for tissues with large diameter functional cells, such as liver, adult heart, or brain (Miltenyi Biotec Debris Removal Kit is recommended for such tissues). For samples with necrosis due to drug treatment, impurities can be separated using the Percoll density gradient centrifugation method.

2.3. High aggregation rate (> 20%):

The aggregation rate of dissociated primary tissues is mostly between 10-20%. If this range meets your experimental requirements, further optimization is not required. However, in the case it does not meet the requirements, a 20-40 μm cell strainer (recommend Flowmi Cell Strainers BAH136800040 from Sigma-Aldrich, which has shown good results) can be used to filter the suspension again.

【Limitations of testing methods】

1. This reagent kit is a sample preparation reagent kit. The dissociated product obtained from sample processing can be used for further in vitro diagnostic tests, however, IT SHOULD NOT BE USED DIRECTLY AS THE RESULT OF AN IN VITRO DIAGNOSTIC TEST.
2. The operators using this reagent kit should possess a certain level of theoretical knowledge and practical skills in molecular biology. Only individuals who have undergone training and obtained qualification are permitted to handle this assay kit.
3. This reagent kit is only applicable to the sample types that are indicated in the【Sample Requirements】.

【Product Performance Indicators】

1. Appearance:

Enzyme H: Clear light orange-red or pink liquid.

Enzyme A, Enzyme R: Clear, transparent liquid.

2. Filling Volume: Should not be lower than the indicated amount.

	Component	8 tests	30 tests*
SeekMate Tissue Dissociation Kit A Pro	Enzyme H	290 µL	1080 µL
	Enzyme A	210 µL	780 µL
	Enzyme R	345 µL	1300 µL

3. Performance:

Taking an appropriate amount of Tissue Dissociation Reagent A Pro for 12% SDS-PAGE electrophoresis, the electrophoresis results show:

There is a clear band between 100 kDa and 130 kDa, and two distinct bands at 25 kDa.

【Precautions】

1. Before use, please read the product manual carefully and strictly follow the instructions provided.
2. After opening the reagents, store them according to the specified storage conditions and use them within their expiration date. It is not recommended to use reagents that have expired.
3. If a single use of tissue amount exceeds 1 g or there is a need to use a dissociation instrument, it is recommended to increase the amount of dissociation solution by 1 mL per 100 mg of tissue based on the standard protocol.
4. During the valid period, if you observe white precipitates in the dissociation solution during the thawing process, simply mix it well, as it will not affect normal usage.
5. After the product is used up, dispose of the waste according to the relevant medical waste disposal guidelines.

【Revised date】

2024/08/05

【Attachment: Detailed recommendations for third-party consumables】

			Product	Ref. No.	Brand	Size	Note
Cell suspension preparation	Reagents	Room temperature	Nuclease-Free Water (not DEPC-Treated)	4387936	Invitrogen		
			1X PBS Buffer	SH30256.LS	HyClone	6 x 1000 ml	Contains no calcium or magnesium
		4℃	RPMI 1640 Medium	11875093	Gibco	500 ml	Recommend
			Fresh Tissue Storage Solution	/	SeekGene	/	Or Miltenyi
			MACS BSA Stock Solution	130-091-376	Miltenyi	/	Cell protectant
			AO/PI Staining Kit	/	SeekGene	/	Cell dyes for fluorescent cell counter
			Red Blood Cell Lysis Buffer	R1010 - 500ml	Solarbio	500 ml	Necessary for tissue dissociation
			Dead Cell Removal Kit	130-090-101	Miltenyi	for 1×109 total cells	Prepared for dead cell removal
			Debris Removal Kit	130-109-398	Miltenyi	2×45 ml	Prepared for debris removal
		-20℃	FBS	10100147C	gibco	500 ml	Cell protectant
			Trypsin 0.25%	SH30042.01	Hyclone	100 ml	Re-digestion for high cell aggregation
	Instruments		Refrigerated Centrifuge		Thermo Fisher	Centrifuge tube: 15/50 ml	Necessary
			Cell Counter (AO/PI)	/	/	/	Fluorescent cell counter recommended
			Microscope	/	/	10/20//40X	Assist in cell quality control
			Vortex	/	/		Mix reagents
			Compact Handheld Centrifuge	/	/	LX-200B	Centrifuge tube: 0.2/1.5 ml
			Ice machine	/	/	IMS-60 (Bin capacity:25kg)	
	RT Consumables		MS Separation columns	130-042-201	Miltenyi	25 columns	Dead cell sorting column
			MAC Smart Strainer (70 μm)	130-098-462	Miltenyi	50 filters (sterile)	Cell filtrator
			MACS Separator		Miltenyi		Magnetic separator
			falcon Cell strainer 40μm	352340	Corning	50 filters	Cell filtrator
			Flowmi Cell Strainers 40 μm	BAH136800040	Sigma-Aldrich	pack of 50 ea	Cell filtrator
General Consumabes			15ml Conical centrifuge tube	430790	Corning		Sterile Rnase-free, Dnase-free
			50ml Conical centrifuge tube	430828	Corning		

			TC-Treated Multiple 6 Well Plates	CLS3516	Corning	case of 50 (individually wrapped)	
			1.5 ml DNA LoBindTub	22431021	Eppendorf	NA	Sterile Rnase-free, Dnase-free; Lobind
			2.0 ml DNA LoBind Tub	22431048	Eppendorf	NA	
			5.0 ml Tubes (with lid)	30119487	Eppendorf	NA	
			75% Medical alcohol	/	/	500 ml	
			Scissors and Tweezers	/	/		