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SeekMate FFPE Dissociation Kit

USER MANUAL

V1.0

K02301-0801 & K02301-0802 & K02101-0806 & K02301-0803

Envision the Future

Beijing SeekGene BioSciences Co.,Ltd

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1. Introduction

Product Name: SeekMate FFPE Dissociation Kit

Specifications: 8 test per set

Intended Use

This product is intended for the isolation of cell nuclei from Formalin-Fixed and Paraffin-Embedded (FFPE) samples. It is for research use only and not for therapeutic purposes.

Principle of Assay

The process involves deparaffinization of FFPE tissue slices, followed by a series of gradient rehydration steps and cell membrane disruption, ultimately exposing the cell nuclei.

Main Components

The SeekMate FFPE Dissociation Kit (K2301-08) includes the following components, each with specific functionalities and storage conditions: SeekMate FFPE Nuclei Isolation Kit, SeekOne™ DD Single Cell Decrosslinking Kit, SeekMate FFPE Pre-decrosslinking Kit, and SeekMate Fixed Cell/Nuclei Storage Kit.

Name & PN & Storage	Quantity	Tube lid color	Component	CN	8 tests/set
SeekMate FFPE Nuclei Isolation Kit V1.0 K02301-0801, store at **2-8℃**	1		NLB	R0012901	10 mL
			WB1	R0013001	50 mL
			WB2	R0013101	10 mL
SeekMate FFPE Pre-decrosslinking Kit V1.0 K02301-0802, store at **-20 ± 5℃**	1		Enzyme K2	R0013201	18 μL
			RNase Inhibitor	R0011903	140 μL
SeekOne™ DD Single Cell Decrosslinking Kit V1.0 K02101-0806, store at **Room temperature**	1		Buffer S	R0012501	0.5 mL
			Buffer T	R0012601	0.5 mL
			DCL buffer	R0012301	0.5 mL×2 tubes
			Enzyme K1	R0012401	90 μL×2 tubes
SeekMate Fixed Cell/Nuclei Storage Kit V1.0 K02301-0803, store at **-20 ± 5℃**	1		Buffer Q	R0013301	0.5 mL×8 tubes
			WB3	R0013401	10 mL

*Note: * The "SeekMate Fixed Cell/Nuclei Storage Kit" is used for short-term storage of isolated cell nuclei. In case further experiments are not performed directly after dissociation, the resulting cell nuclei can be stored using this kit. Refer to Appendix 1 for detailed instructions for use.*

Storage Condition

Name	PN	Transportation	Storage
SeekOne™ DD Single Cell Decrosslinking Kit V1.0	K02101-0806	Ambient	Room temperature
SeekMate FFPE Nuclei Isolation Kit V1.0	K02301-0801	Ambient	2-8℃
SeekMate FFPE Pre-decrosslinking Kit V1.0	K02301-0802	Dry ice	-20 ± 5℃
SeekMate Fixed Cell/Nuclei Storage Kit V1.0	K02301-0803	Dry ice	-20 ± 5℃

Validity Period

The shelf life of the reagents is 12 months.

Sample Requirements

A. Applicable Sample Types

This kit is suitable for formalin-fixed paraffin-embedded (FFPE) tissue slices.

B. Sample Collection

B.1 Avoid sampling necrotic, calcified, sclerotic, or fibrotic areas, especially in diseased tissues.

B.2 For tissues cut with an electrocautery, remove any necrotic tissue resulting from electrocautery contact.

B.3 Ensure target tissue is free of ice crystals at the time of sampling.

B.4 After sampling, trim the tissue and quickly remove non-target areas before embedding to prevent affecting the final sequencing results.

B.5 Consider the experimental model in advance to assess its impact on the cells.

B.6 Consider any prior drug treatments to evaluate their effect on the cells.

B.7 **During sample preparation, fix tissues in 10% neutral formalin at room temperature for more than 24 hours; ensure tissue slices are larger than 0.5 cm².**

C. Sample Storage

FFPE tissue blocks and slices should be stored at no higher than room temperature conditions.

D. Sample Transport

Transport at room temperature.

Self-supplied instruments and reagents

Name	Recom. Model	Recom. Manufacturer and Item No.
Xylene	1 L	Millipore Sigma, 214736
Ethanol absolute	500 mL	Sangon Biotech, A500737
Double distilled water	500 mL	Sangon Biotech, A500197
PBS Buffer	500 mL	Sangon Biotech, E607008
VAHTS DNA Clean Beads	450 mL	Vazyme, N411-03
4% Paraformaldehyde Fix Solution	500 mL	Sangon Biotech, E672002-0500
NGS™ RNA HS Assay Kit	1,000 assays	ABP；FP008
Qubit™ RNA High Sensitivity (HS) Assay Kits	500 assays	Thermo；Q32855
KIMBLE Dounce tissue grinder set	2 mL; 7 mL	Kimble® 885300-0002; Kimble® 885300-0007
Sterile Cell Filters	20 μm	pluriStrainer® 20μm(Cell Strainer), 43-50020-03
	30 μm	MACS® SmartStrainers (30μm), 130-110-915
Scissors	Scissors (10 cm, Straight, Sharp)	/
Cell counter	CountStar Rigel S2	Countstar, IN030101
	SeekMateTinitan™ Fluorescence Cell Counter	SeekGene, M002C
Magnetic separator	24-well x 200 μL	Mich Scientific, Magpow-24
	16-well x 1.5 mL	Invitrogen, 12321D
Pipettes	0.1-2.5 μL, 0.5-10 μL, 2-20 μL, 20-200 μL, 100-1,000 μL	Eppendorf, -; RAININ, -
Thermal cyclers capable of uniformly heating 100 μL emulsion volumes	C1000 Touch™ Thermal Cycler with 96-Deep Well Fast Reaction Module	BioRad, 1851197
	MasterCycler® Pro	Eppendorf, North America 950030010 International 6321 000.019
	Veriti 96-Well Thermal Cycler	Thermo Fisher, 4375786
	LongGene, A300	LongGene, A300
DNA/RNA quality control equipment	Agilent 4200 TapeStation	Agilent, G2991AA
	Agilent 2100 Bioanalyzer	Agilent, G2939BA
	Bioptic, Qsep400	Bioptic, Qsep400
High Sensitivity RNA ScreenTape	112 reactions	Agilent,5067-5579
High Sensitivity RNA ScreenTape Sample Buffer	500 μL	Agilent,5067-5580
PCR tubes	0.2 mL	Axygen, PCR-02-L-C
	0.5 mL	Axygen, PCR-05-C
8-strip PCR tubes	0.2 mL	Axygen, PCR-0208-FCP-C
centrifuge tubes	5mL; 15mL; 50mL	Axygen
Qubit 4.0	Qubit 4.0 Fluorometer	Thermo Fisher Scientific, Q33238
Mini centrifuge	-	TIANGEN, OSE-MP25
Vortex mixer	IKA Shakers MS3 (MS3.4/MS3.5)	IKA, -
Thermo shaker	TCS10	Hangzhou Ruicheng Instrument Co., Ltd
DNase/RNase-free Low- retention microcentrifuge tubes	1.5 mL	Axygen, MCT-150-L-C
Low retention pipette tips	0.5 - 10 μL 1 - 200 μL 100 - 1000 μL	Axygen, T-300-L-R-S, Axygen, T-200-C-L-R-S, Axygen, T-1000-C-L-R-S

2. Experimental Procedure

Section 1 Nuclei Isolation

Pre-Experiment Preparation

A. Add RNase Inhibitor (1 μL per 1 mL reagent) to the reagents used in the extraction process (NLB, WB1, and WB2) before the experiment.

B. Preheat the thermo shaker to 55°C and 20°C as needed.

C. Prepare 95% ethanol, 80% ethanol, 70% ethanol, 50% ethanol, and 30% ethanol fresh for use (prepare on the day of use, and do not store for more than 24 hours).

D. If not specified, perform operations on ice.

1.1. Deparaffinization and rehydration

1.1.1 Deparaffinization

a. Place 1-2 FFPE tissue slices (50 μm each) into a 1.5 mL centrifuge tube.

b. Add 2 mL of xylene and incubate at room temperature for 10 min to deparaffinize. During deparaffinization, invert the centrifuge tube occasionally to mix.

c. Remove the xylene and repeat the deparaffinization process to ensure complete removal of paraffin.

1.1.2 Rehydration

Rehydrate the tissue at room temperature using a gradient of alcohols as follows:

a. After removing xylene, add 2 mL of 100% ethanol, invert to mix, and incubate for 1 minute. Remove the supernatant after a brief centrifugation and repeat the washing step.

b. Sequentially incubate with 2 mL of 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, and ddH_2O, each for 1 minute, and remove the supernatant after each incubation to complete the rehydration process.

Note 1: Before using the microtome, clean with 70% ethanol and RNAzap solution. Deparaffinization should be completed in a fume hood.

Note 2: For very fragile or small tissue samples, centrifuge briefly before removing the liquid, and then aspirate the solution.

Note 3: After deparaffinization and rehydration, tissue slices can be stored for a short time in 70% ethanol and kept on ice until further use.

Note 4: If the tissue slice area is less than 0.5 cm^2, consider adding 1-2 additional slices for deparaffinization and subsequent washing steps.

Note 5: If white paraffin remains after two treatments with xylene, repeat the deparaffinization process to ensure complete removal.

1.2. Nuclei isolation

1.2.1 After rehydration, remove ddH_2O, and add 1 mL of NLB. Transfer the mixture to a glass grinding tube of the Dounce tissue grinder set (885300-0007, KIMBLE).Use the A rod to perform 10-20 up-and-down strokes, followed by 10-25 up-and-down strokes with the B rod. Incubate on ice for 5 minutes.

Note 1: Start timing when grinding begins. Increase the number of strokes for challenging samples if needed.

Note 2: For larger tissues, initially add 100 µL NLB and use pre-chilled scissors to cut the tissue into smaller pieces. Then, add 900 µL NLB, mix by pipetting, and transferred to the glass grinding tube.

Note 3: The desired grinding state is when the A rod reduces large tissue pieces to smaller fragments and the B rod converts them into a white, opaque suspension.

1.2.2 After incubation, add 1 mL WB1 to the tube and gently mix. Filter using a 20 µm or 30 µm sterile cell strainer.

Note: Rinse the cell strainer with WB1 before use.

1.2.3 Rinse the cell strainer with 2 mL of WB1.

1.2.4 Mix and centrifuge the filtered nuclear solution at 4℃, 1000g for 5 min. Discard the supernatant.

1.2.5 Resuspend the nuclear pellet in 1 mL of WB1. Mix well and perform nuclei counting by the fluorescence cell counter.

1.2.6 Incubate on ice for 5 min, mix the nuclear solution, centrifuge at 4°C, 1000g for 5 min. Discard the supernatant.

Note: If the total number of nuclei counted in the previous step is <200,000, adjust the centrifugation speed to 2000 g.

1.2.7 Resuspend the nuclear pellet in 120 µL of WB2. Mix well and perform nuclei counting by the fluorescence cell counter.

Note 1: If used in conjunction with the SeekOne™ DD FFPE Single Cell Transcriptome-seq Kit (K02101-08; K02101-02), proceed to Section 2 and 3. For other uses, please conduct your own tests.

Note 2: If no further experiments will be performed within 2 hours of this step, you can use the "SeekMate Fixed Cell/Nuclei Storage Kit" to store the samples at -80℃ for up to 30 days. Refer to Appendix 1 for detailed usage instructions.

Section 2 RNA Extraction and Quality Control

2.1. RNA Extraction

2.1.1 Transfer 20,000 cell nuclei from step 1.2.7 into a new 1.5 mL centrifuge tube. Add WB2 to a final volume of 25 µL, then add 25 µL of DCL buffer and 10 µL of Enzyme K1 (totaling 60 µL). After thorough mixing, incubate the mixture at 55°C for 15 min, and then at 80°C for 15 min in a thermo shaker.

Note: If the concentration of cell nuclei is high (＞ 5,000 nuclei/µL), adjust the volume of cell nuclei and dilute with WB2 to achieve a concentration of 2,000-3,000 nuclei/µL for accurate sampling and quality control.

If the concentration of cell nuclei is low (＜ 800 nuclei/µL), use the same volume of DCL buffer as the volume of the 20,000 cell nuclei suspension, keeping the amount of Enzyme K1 unchanged.

If DCL buffer appears as white solids or crystals, heat at 55°C until the solution becomes clear before use.

2.1.2 After incubation, briefly centrifuge the tube and add 120 µL of DNA Clean beads (2×). Vortex thoroughly to mix.

2.1.3 Allow the mixture stand at room temperature for 10 min, then centrifuge briefly and place it on a magnetic rack to adsorb until the solution becomes clear. Remove and discard the supernatant carefully.

Note: During adsorption, use a pipette tip to gently pipette up and down 5 times to mix and enhance magnetic bead adhesion, tilt the tip away from the beads.

2.1.4 Add 800 µL of 80% ethanol while maintaining the tube on the magnetic rack. After about 30 seconds, carefully remove and discard the supernatant. Repeat this step once.

2.1.5 Close the lids and briefly centrifuge them. Put the tubes back onto the magnetic stand, and **remove any residual supernatant with a 10 µL pipette**. During centrifugation, ensure that the side of the tubes with the beads is facing away from the central axis.

2.1.6 Keep the tubes open and allow the ethanol to evaporate for about 3-5 minutes (depending on the environmental conditions, the beads will be dark and matte without drying or cracking, do NOT wait too long) by incubating at room temperature. Add 15.5 µL of nuclease-free water to fully suspend the beads, vortex for 10-15 seconds, then briefly centrifuge before pipetting up and down 15 times. Incubate at room temperature for 2 minutes on a normal rack (not on the magnetic stand) and then briefly centrifuge.

2.1.7 Place the tubes onto the magnetic rack until the beads are stuck to the magnet and the solution appears clear, **transfer 15 µL of supernatant to a new 0.2 mL PCR tube**.

2.2. RNA Quality Control

Perform total RNA quantification (detecting sample concentrations of 1-10 µL) and nucleic acid fragment analysis. Determine whether to proceed with subsequent experimental steps based on the remaining cell nucleus quantity and quality control results.

2.3. Quality Control Criteria:

#	RNA QC results (20,000 nuclei)				Result
	total RNA (QUBIT)	Main peak(4150/4200)	DV200	Amount of nuclei remaining	
1	>25 ng ☑	>200 bp ☑	>40% ☑	≧350,000 ☑	**Pass**
2	>25 ng ☑	>200 bp ☑	>40% ☑	50,000-350,000 ☑	**Risk**
3	>25 ng ☑	>200 bp ☑	>40% ☒	≧50,000 ☑	
4	>25 ng ☑	>200 bp ☒	>40% ☑	≧50,000 ☑	
5	10-25 ng ☑	>200 bp ☑	>40% ☒	≧50,000 ☑	
6	10-25 ng ☑	>200 bp ☒	>40% ☑	≧50,000 ☑	
7	10-25 ng ☑	>200 bp ☑	>40% ☑	≧50,000 ☑	
8	/	/	/	<50,000 ☑	**Failure**
9	<10 ng ☑	/	/	/	
10	/	>200 bp ☒	>40% ☒	/	

Section 3 Sample Pre-decrosslinking

3.1. Reagent Preparation:

Thaw WB3 and RNase Inhibitor on ice for 30 minutes before use. Prepare the resuspension buffer according to the table below for a single sample.

Component	Resuspension Buffer (final conc. 50U/mL)
1×PBS	1 mL
RNase Inhibitor	1.25 μL
Total	~1 mL

3.2. Pre-decrosslinking

3.2.1 Use WB2 to adjust the cell nucleus suspension from step 1.2.7 to a concentration of 3,500-5,000 nuclei/μL. Take 100 μL of the cell nucleus suspension, add 2 μL Enzyme K2, 1 μL RNase Inhibitor, and 4 μL Buffer S. After thorough mixing, incubate the mixture at 20°C for 15 min, followed by incubation at 80°C for 45 min.

Note: Due to sample variation, this step is recommended to use 350,000-500,000 cell nuclei for. If the nucleus count is between 50,000-350,000, keep the processing conditions unchanged.

3.2.2 After incubation, centrifuge at 4°C, 2,000g for 5 min to pellet the cell nuclei. Remove the supernatant, resuspend the cell nuclei in 200 μL of WB2, pipette and mix well, and perform cell nucleus counting.

3.3. Nuclei Fixation

3.3.1 Add the appropriate volume of 4% paraformaldehyde solution to the remaining nuclei to achieve a final concentration of approximately 2.5%. Pipette and mix well and fix at room temperature for 10 min.

Note: to fix 75 µL of nucleus suspension, add 25 µL of 4% paraformaldehyde solution (active component 4%) to ensure the effective concentration is around 1%.

3.3.2 After fixation, add 1 mL of WB3, pipette and mix well, centrifuge at 4°C, 1,000g for 5 min, and remove the supernatant.

3.3.3 Resuspend the pellet in 1 mL of WB3, pipette and mix well, centrifuge at 4°C, 1000g for 5 min, and repeat this washing step once.

3.3.4 After removing the supernatant, resuspend the nucleus pellet with resuspension buffer, pipette and mix well, and perform nuclei counting by fluorescence cell counter.

Note: The recommended resuspension concentration is 1,500-2,000 nuclei/µL. Adjust the resuspension volume according to the expected concentration.

3.3.5 Store the cell nuclei on ice or at 4°C for later use (**within 30 min for the next step**).

Note: If no further experiments will be conducted after this step, you can use the SeekMate Fixed Cell/Nuclei Storage Kit to store at -80°C for up to 7 days. See Appendix 1 for detailed usage instructions.

3. Precautions

A. After opening, store the reagents according to the specified storage conditions and use them within the expiration date. Expired reagents are not recommended for use.

B. During the validity period, if white precipitate appears in NLB, mixing it will not affect normal use.

C. Dispose of waste products according to relevant medical waste disposal regulations after use.

D. The results of this kit can be influenced by factors such as the source of the sample, the sample collection process, sample quality, transportation conditions, and sample preprocessing. Additionally, factors such as nucleus quality and operating environment may affect the results. Users must be aware of potential errors and limitations in accuracy during the testing process. This kit should only be used by trained personnel with appropriate laboratory skills.

Appendix 1 SeekMate Fixed Cell/Nuclei Storage Kit Instructions

Intended Use

This reagent kit is designed for the preservation of cell nuclei obtained from formalin-fixed paraffin-embedded (FFPE) tissues, as well as from cells or cell nuclei fixed with formaldehyde.

1.Usage

1.1. Cell Nuclei Dissociated from FFPE Samples

1.1.1 For nuclei obtained directly from FFPE samples (nucleus suspension from step 1.2.7)

Centrifuge the cell nuclei suspension at 4°C, 1000 g for 5 minutes and remove the supernatant. Resuspend cell nuclei in 400 µL of Buffer Q (recommended for up to 50,000 cell nuclei), mix well, and **store at -80℃ for up to 30 days.**

1.1.2 For nuclei after Pre-decrosslinking and Fixation (nucleus suspension from step 3.3.5):

Centrifuge the cell nuclei suspension at 4°C, 2000 g for 5 minutes and remove the supernatant. Resuspend cell nuclei in 400 µL of buffer Q (recommended for up to 500,000 cell nuclei), mix well, and **store at -80℃ for up to 7 days.**

1.2. Cell or Cell Nuclei Fixed with Formaldehyde

For cells or nuclei fixed with formaldehyde, centrifuge at 4℃, 1000g for 5 minutes, and remove the supernatant. Resuspend cell nuclei in 400 µL of Buffer Q (recommended for up to 50,000 cell nuclei), mix well, and **store at -80℃ for up to 30 days.**

2. Post-Preservation Handling:

After thawing the stored nucleus solution completely, add 800 µL of WB3, mix well, centrifuge at 4℃, 1000g for 5 minutes. Remove the supernatant. Resuspend in 0.2 mL of WB3, mix well, and perform cell nucleus counting. Store on ice until further use.

3.Disposal Instructions

Dispose of waste according to relevant medical waste disposal regulations after using the product.

Appendix 2: Manufacturer and EU representative information

Manufacturer/After-sales service unit

Manufacturer: Beijing SeekGene BioSciences Co.,Ltd

Address: Room 201, Floor 2, Tower A Building 9, Zone 1, 8 Life Science Parkway, Changping District, Beijing, China

Zip code: 102206

Tel: +86- (0) 10 56918048

Information on EU representatives

EU Name: Medpath GmbH

EU Address: Mies-van-der-Rohe-Strasse 8,80807 Munich, Germany

DIMDI No: DE/0000047823

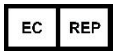
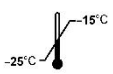
SRN Code: DE-AR-000000087

Tel: +49 (0) 89 189174474

Fax: +49 (0) 89 5485 8884

Email: info@medpath.pro

Appendix 2: Explanations for Symbols

	Manufacture		European union representative
	In vitro diagnostic medical device		Use-by date
	Batch code		Catalogue number
	Unique device identifier		Consult instructions for use
	Keep dry		Keep away from sunlight
	Do not use if package is damaged and consult instructions for use		Cautions
	Biological risks		Fragile, handle with care
	Store at -25~-15℃		CE mark
	Store at 2~8℃		Store at 0~25℃

Appendix 3: Revision

No.	Revision	Modified Content	Effective Date
1	New Creation	New file	2024/04/01
2	Revision	Revision	2024/06/14