YamayBio

Tissue ExM Kit

QUICK START GUIDE

For research use only.
Not for use in diagnostic procedures.

Contents and Storage

Tissue ExM Kit

CC6234 20 Assays

Storage:
Store at 4 °C for 12 months. Transported at room temperature.

Component	Cat. No.	Qty
ExMPro Reagent A Plus	CC6209	6 mL
ExM Reagent B (100×)	CC6202	60 µL
ExM Reagent C (100×)	CC6203	60 µL
ExMPro Reagent D (1,000×)	CC6210	60 µL
ExMPro Reagent E	CC6211	60 mL
Casting Frame	CC6212	20
Stickers	CC6213	20

Introduction

Expansion microscopy (ExM) is a super-resolution imaging method in which biological samples are isotropically expanded by swellable hydrogels. This enables nanoscale imaging using conventional wide-field and standard confocal microscopes. The method is compatible with proteins, nucleic acids, and lipids. The Tissue ExM Kit is validated for use with FFPE tissue sections or frozen tissue sections and enables uniform three-dimensional expansion of approximately 4~6-fold, corresponding to an effective 4~6-fold increase in spatial resolution. The isotropic expansion preserves the spatial distribution of biomolecules and provides advantages such as reduced cost and increased imaging depth compared to purely optical super-resolution techniques.

Pre-Experiment Guidelines

- **Applicable tissue types:** suitable for relatively soft tissue samples
- **Validated tissues:** brain, placenta, liver, kidney, fetus, testis, pancreas, and colorectum tissues
- **Not applicable to:** hard tissues such as bone, dentin, enamel, or cartilage
- **Antibody optimization** (relative to standard dilution conditions):
  - Fluorescent secondary antibodies: standard dilution of 1:1000 → approximately 1:100–1:200
  - Primary antibodies: standard dilution 1:500 → approximately 1:50–1:100
- **Signal requirement before expansion:**
  - Before proceeding with the expansion protocol, ensure that the fluorescence signal is bright and near-saturated under maximum excitation.
- **Additional materials required:**
  - DAPI solution (1 μg/mL prepared in ddH_2O)
  - Dark Chamber
  - 5 mL or 15 mL centrifuge tubes
  - Glass-bottom dish or microscope slide
  - Inverted Fluorescence Microscope/ Confocal/ SIM / STED/ STORM

Quick Protocol

Perform immunofluorescence staining according to standard protocols. Once stained, proceed with the expansion procedure described below.

1

Mix gel solution

1. Gel Preparation:

Prepare the gel solution according to Table 1.

Table 1. Reagent volumes for gel solution (per sample)

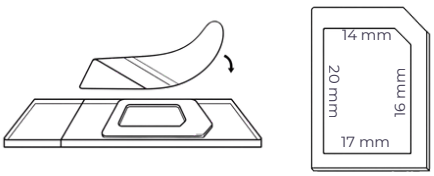
Reagents	Volume
ExMPro Reagent A Plus	294 μL
ExM Reagent B (100×)	3 μL
ExM Reagent C (100×)	3 μL

*A total of 300 μL of the reaction mixture is generally sufficient to cover sample fully.

2. Gel Embedding:

Peel off the backing film from the **casting frame** and attach it to a microscope slide. Add **300 µL** of gel solution to the inner frame, then cover with the **sticker**, and incubate at 4 °C in the dark for 1 hour. Transfer to a 37 °C incubator and continue incubating in the dark for **another 1 hour**.

2



- **Incubate at 4°C in the dark for 1 h**
- **Incubate at 37°C in the dark for another 1 h**

3. Homogenization:

Add 3 mL of **ExMPro Reagent E** to a 5 mL or 15 mL centrifuge tube, then add 3 µL of **ExMPro Reagent D (1,000×)** and mix thoroughly. Completely immerse the gel sample in the solution, cap the tube, and incubate at **37°C in the dark for 1–2 h** (1 h for brain tissue sections; 2 h for placenta, liver, kidney, embryo, colorectal, and other tissue sections).

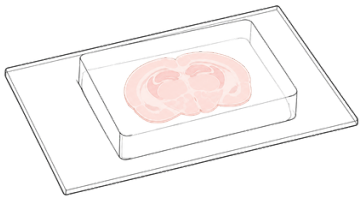
3

- **Mix homogenization reagent**
- **Incubate at 37°C in the dark for 1–2 h**

4. Expansion:

Wash the homogenized gel **twice** with ddH_2O. Transfer the gel to approximately 100 mL of fresh ddH_2O. Allow the gel to expand **in the dark** at room temperature for **6 hours** or **at 4 °C overnight**.

4



Expand in the dark at room temperature for 6 hours or at 4 °C overnight

5

Imaged directly with an inverted fluorescence microscope

5. Imaging:

Discard the excess water and carefully remove the expanded gel. Restain the gel with **DAPI solution** (1 μg/mL prepared in ddH_2O) for **2 minutes**, then rinse with ddH_2O **3 times (5 minutes each)** to remove excess DAPI.

Cut it into manageable sections, and transfer to a glass-bottom culture dish. Add a small amount of ddH_2O to keep the sample moist and to prevent it from floating.

Position the gel with the **tissue-containing side facing down** onto a glass-bottom dish or microscope slide (No cover slipping is required).

Image the expanded gel directly using an **inverted fluorescence microscope**.

Note:

1. Glass-bottom culture dishes are recommended for imaging with oil-immersion objectives. Ensure the gel remains hydrated when using air objectives.
2. During expansion, DAPI dissociates from nucleic acids. If nuclear staining is required, re-stain the expanded gel with DAPI in ddH_2O for 5 minutes, followed by washing with ddH_2O. Do not use PBS, as this will cause gel shrinkage.

Troubleshooting

Experimental Stage	Issue	Possible Cause	Solution
Sample Preparation	Weak or no fluorescence signal	Antibody concentration may be too low or staining conditions may not be optimal	Optimize antibody concentration and staining conditions before expansion. See the pre-experiment guidelines for recommended antibody optimization
Gel Preparation & Embedding	Bubbles in the gel	Improper sealing during gel assembly	Pipette gel solution into the casting frame and gently lower the stickers from one side to remove bubbles
		Insufficient gel solution	Prepare enough gel solution and fill the casting chamber completely according to the recommended protocol
	Gel leakage	Incomplete sealing of the casting chamber	Before pipetting, check whether the bottom sticker is tightly attached. After pipetting, gently lower the top sticker from one side to remove bubbles and ensure proper sealing
	Gel does not polymerize or remains liquid	Polymerization time was too short	Allow the gel to polymerize completely before proceeding to the next step
		Incorrect preparation of the polymerization mixture	Prepare all reagents according to the recommended mixing ratios
		Contaminated or expired reagents/consumables	Use clean laboratory consumables and freshly prepared reagents

Troubleshooting

Experimental Stage	Issue	Possible Cause	Solution
Expansion	Gel tearing during removal after polymerization	Stress concentration points form around the sample; forcible tearing will rupture the gel block.	Wet the gel with ExMPro Reagent E to facilitate smooth and intact removal.
	Gel cracks during expansion	Incomplete gel polymerization	Ensure the gel has fully polymerized before initiating expansion
	Insufficient or uneven expansion (thickness not uniform)	Expansion time may have been insufficient	Allow the gel to expand fully under the recommended conditions before imaging
		Impure water used during expansion	Use freshly prepared ultrapure water throughout the expansion process
	DAPI signal loss	DAPI is not covalently anchored and may diffuse out during expansion	Restain the expanded gel with DAPI (prepared in ultrapure water) for approximately 2 minutes before imaging

Troubleshooting

Experimental Stage	Issue	Possible Cause	Solution
Imaging	Blurred or out-of-focus images	Improper mounting or imaging setup.	Use an imaging chamber, coverslip, and immersion medium compatible with expanded samples
	Weak or no fluorescence signal after expansion	The initial fluorescence signal was too weak before expansion	Repeat the staining procedure and optimize antibody labeling before expansion
		Fluorescence signal decreased during expansion	Optimize antibody concentration and fluorophore selection according to the pre-experiment guidelines
		Selected fluorophore is not compatible with ExM	Alexa Fluor®, ATTO®, and DAPI are generally recommended because they retain fluorescence well after expansion
	Gel movement during imaging	Excess liquid surrounding the expanded gel	Keep the gel hydrated using only a minimal amount of ultrapure water to reduce sample movement
	Uneven focus across the expanded sample	Expanded gels have increased thickness, resulting in multiple focal planes	Acquire Z-stack images and reconstruct the final image using image-processing software when necessary
	Signal decreases after prolonged imaging	Photobleaching	Minimize exposure to high-intensity excitation light by reducing illumination time and intensity whenever possible
Post-expansion Sample Storage	Image quality decreases after storage for several days	Prolonged storage after expansion	Store expanded samples in ultrapure water at 4°C in the dark for up to 3–5 days. For optimal image quality, perform imaging as soon as possible after expansion