YamayBio

Cell ExM Kit

QUICK START GUIDE

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

Contents and Storage

Cell ExM Kit
CC6232A 20 Assays

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

Component	Cat. No.	Qty
ExM Reagent A	CC6201A	6 mL
ExM Reagent B (100×)	CC6202	60 µL
ExM Reagent C (100×)	CC6203	60 µL
Casting Frame	CC6207	2
Stickers	CC6205	30

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 Cell ExM Kit is optimized for immunofluorescence staining of cultured cells and enables uniform three-dimensional expansion of approximately 4.5×, resulting in an effective ~4-fold increase in spatial resolution (from ~250 nm to ~60 nm with wide-field microscopy, and from ~120 nm to ~30 nm with confocal microscopy). Isotropic expansion preserves the spatial distribution of biomolecules while offering reduced cost and increased imaging depth compared to optical super-resolution imaging techniques.

Pre-Experiment Guidelines

- **Applicable cell types:** Animal cells
  - Adherent cells: ≥1 × 10^5 cells per sample
  - Suspension cells: >1 × 10^5 cells per sample
- **Not applicable to:** Plant cells or cells with cell walls
- **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:**
  - Cell culture coverslips
  - Dark chamber
  - Inverted fluorescence microscope/ confocal microscope/ SIM/ STED/ STORM

Quick Protocol

Perform immunofluorescence staining according to standard protocols. After staining, proceed with the expansion workflow described below.

1

Mix gel solution

1. Gel Preparation:

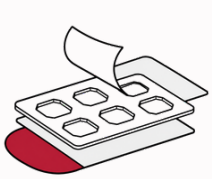
Prepare the gel solution according to Table 1.

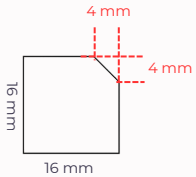
Table 1. Reagent volumes for gel solution (per sample)

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

*A total volume of 300 μL is generally sufficient to completely cover the sample in one well.

2



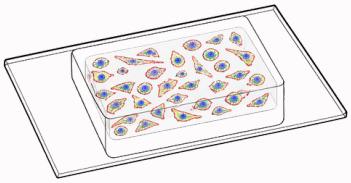


- **Add 300 µL of gel buffer per well**
- **Incubate in the dark for 45 minutes**

2. Gel Embedding:

Peel off the sticker and firmly attach the adhesive side to the bottom of the casting frame. Place the cell culture coverslips into the casting frame. Add **300 µL of gel solution to each well** and cover the samples with a new adhesive sticker. Incubate in **the dark for 45 minutes.**

3



Expand 2 hours or at 4 °C overnight

3. Expansion:

Remove the stickers and carefully separate the gel from the cell culture coverslips. Allow the gel to expand **in the dark** at room temperature for **2 hours or at 4 °C overnight**.

4

Imaged directly with an inverted fluorescence microscope

4. Imaging:

Discard the excess water and cut the gel into manageable sections. Add a small amount of ddH_2O to keep the gel hydrated and to prevent flotation.
Position the gel with **the cell containing side facing down** (the side with the coverslip impressions) onto a glass-bottom dish or microscope slide (**No coverslip is required**). Image the expanded gel directly using an inverted fluorescence microscope or other compatible imaging system.

Note:

- Glass-bottom culture dishes are recommended for imaging with oil-immersion objectives. Ensure the gel remains hydrated when using air objectives.
- During the expansion process, the cell culture coverslip will separate from the gel, as the cell samples have already been transferred into the gel.
- During expansion, DAPI dissociates from nucleic acids. If nuclear staining is required, re-stain the expanded gel with DAPI prepared in ddH_2O for 2 minutes, followed by rinsing with ddH_2O 3 times (5 min each) to remove excess DAPI dye. Do not use PBS, as this will cause gel shrinkage.

Troubleshooting

Experimental Stage	Issue	Possible Cause	Solution
Sample Preparation	Cells detach or do not adhere well	Adequately fixed before processing	Remove non-adherent cells, fix adherent cells according to the protocol, and allow the coverslip dry completely at 37°C before proceeding
		Improper storage after sample drying	Store after dried samples as recommended in the protocol and rinse gently with PBS before continuing the experiment
	Poor or incomplete expansion	Cells contain rigid cell walls (plant/bacterial cells) that prevent efficient expansion	Remove cell wall (e.g., generate protoplasts) before performing ExM
	Low cell density	Cell confluency is too low before fixation	Increase the seeding density to achieve approximately 60–80% confluency before fixation
	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

Troubleshooting

Experimental Stage	Issue	Possible Cause	Solution
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 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