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.

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 type:** 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: from standard 1:1000 → ~1:100–1:200
  - Primary antibodies: from standard 1:500 → ~1:50–1:100
- **Signal requirement before expansion:** Fluorescence signal must reach a sufficiently high intensity (bright, near-saturated under maximum excitation) before proceeding to expansion procedures
- **Additional Material Required:**
  - Cell Culture Coverslips
  - Dark Chamber
  - Inverted Fluorescence Microscope/ Confocal/ 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

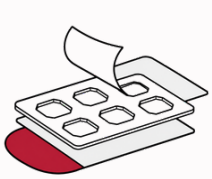
1. Gel Preparation:
Prepare the gel solution according to Table 1.

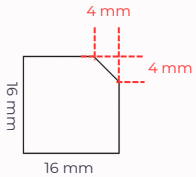
Table 1. Reagent volumes for gel solution (per sample)

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



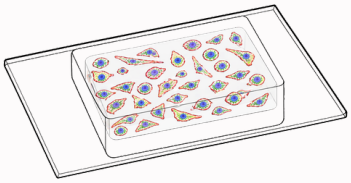


- **Add 300 µL of gel buffer per well**
- **Incubate in the dark for 45 minutes**

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 the non-adhesive side of the sticker. Incubate in **the dark for 45 minutes.**

3



Expand 2 hours or at 4 °C overnight

3. Expansion:

Remove the sticker 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.