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. / Size	Qty
ExM Buffer A	CC6201A	6 mL
ExM Buffer B	CC6202	60 µL
ExM Buffer C	CC6203	60 µL
Plastic sheet	CC6207 / 16 × 16 × 1 mm	2
Stickers	CC6205	30
Glass Bottom Dishes	CC6206	4

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-fold, corresponding to 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). 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.

Additional Material Required

1. Cell Culture Coverslips
2. Dark Chamber
3. 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.

Note: Use the original fluorescent antibody at 4–8× its standard concentration.

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 Buffer A	294 µL
ExM Buffer B	3 µL
ExM Buffer C	3 µL

*A total of 300 µL of the reaction mixture is generally sufficient to cover one 16 × 16 × 1 mm well with the sample fully.

2

- **Add 300 µL of gel buffer per well**
- **Incubate in the dark for 30 minutes**

2. **Gel Embedding**:
   Peel off the sticker and firmly attach the adhesive side to the underside of the plastic sheet. Place the φ 9 mm /φ 15 mm cell culture coverslips into the corresponding 10 mm and 15 mm wells. Add **300 µL of gel buffer per well** and cover the samples by placing the non-adhesive side of the sticker onto the plastic sheet. Incubate in **the dark for 30 minutes.**

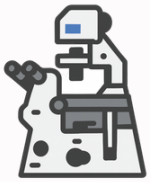
3

Expand 2 hours or at 4 °C overnight

3. **Expansion**:

   Remove the sticker and place the gel containing the cell culture coverslips into excess water. 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 carefully remove the expanded gel, 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 avoid adding too much to prevent the gel from floating. Position the gel with the cell-containing side facing down (the side with the coverslip impressions) onto microscope slide. 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 the expansion process, the cell culture coverslip will separate from the gel, as the cell samples have already been transferred into the gel.
3. During gel expansion, DAPI dissociates from nucleic acids. If nuclear staining is required, re-stain the expanded gel with DAPI prepared in ddH_2O for 5 minutes, followed by washing with ddH_2O. Do not use PBS, as this will cause gel shrinkage.