YamayBio

Cell ExM Kit

QUICK START GUIDE

Research use only.
Not for diagnostic procedures.

Contents and storage

Cell ExM Kit

CC6232 20 Samples

Storage:
Store at 4 °C for 12 months.
Store ExM Buffer D at –20 °C when not in use for extended periods.

Component	Cat. No. / Size	Qty
ExM Buffer A	CC6201	4 mL
ExM Buffer B	CC6202	4 mL
ExM Buffer C	CC6203	40 uL
ExM Buffer D	CC6204	40 uL
Plastic sheet	φ10 mm / φ15mm	2
Stickers	CC6206	20

Introduction

Expansion microscopy (ExM) is a super-resolution imaging method in which biological samples are isotropically expanded by swellable hydrogels. This approach enables high-resolution imaging with conventional wide-field microscopes and super-resolution imaging with standard confocal microscopes. The method is compatible with proteins, nucleic acids, and lipids. This product has been optimized for immunofluorescence staining of cultured cells, allowing uniform three-dimensional expansion of approximately 4.5-fold, which corresponds 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 coverslip
2. dark chamber
3. inverted fluorescence microscope/ Confocal/ SIM / STED/ STORM

Quick Protocol

Experiment following the immunofluorescence protocol. Once fluorescently stained cell-seeded coverslips are obtained, continue with the procedure.

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

1. **Fixation:**
   Rinse the circular coverslips in the cell culture plate with ddH_2O, then **add 200 µL of ExM Buffer A** onto the samples. Incubate at room temperature in the **dark for 30 minutes.** After incubation, remove ExM Buffer A and **wash the coverslips** with ddH_2O, then carefully remove excess liquid.

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

Table 1. Reagent volumes for gel solution

Reagents	Volume
ExM Buffer B	196
ExM Buffer C	2
ExM Buffer D	2

*200 µL of the reaction mixture is generally sufficient to cover one 10 mm/ 15mm well with the sample fully.

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Add 200 µL of gel buffer/ well
Incubate in the dark for 30 minutes

3. **Gel Embedding**:
   Peel off the sticker and firmly attach the adhesive side to the underside of the plastic sheet. Place the φ9 mm /φ15 mm cell culture coverslips into the corresponding 10 mm and 15 mm wells. Add **200 µL of gel buffer to each well**, then cover the samples by placing the non-adhesive side of the sticker onto the plastic sheet. **Incubate in the dark for 30 minutes.**

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expand 2 hours or at 4 °C overnight

4. **Expansion**:
   Remove the sticker and place the gel containing the cell culture coverslips into excess water. Allow the gel to expand at room temperature for **2 hours or at 4 °C overnight**. Discard the excess water, carefully remove the gel sample, cut it into small pieces, and transfer them to a glass-bottom culture dish. Add a small amount of ddH_2O to keep the sample moist and to prevent it from floating.

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imaged directly with an inverted fluorescence microscope

5. **Imaging:**
   No coverslipping is required for the expanded gel. Position the gel with the cell-containing side facing down (the side with the coverslip impressions) and image directly using an inverted fluorescence microscope.

Note:

1. Glass-bottom culture dishes are more suitable for observation under an oil-immersion objective. Make sure the gel stays moist when using an air objective.
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. DAPI will dissociate from nucleic acids during the gel expansion process. Therefore, if nuclear staining is required, the expanded gel should be re-stained with DAPI solution prepared in ddH_2O for 5 minutes, followed by washing with ddH_2O before microscopic observation or imaging. Do not use PBS to prepare the DAPI solution, otherwise, the gel will shrink.