YamayBio

Cell ExM Plus Kit

QUICK START GUIDE

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

Contents and Storage

Cell ExM Plus Kit

CC6233 20 Assays

Storage:
Store at 4 °C for 12 months. Transported at room temperature. After mixing ExMPro Reagent A with Regent A Diluent, store the prepared Solution A at −20°C for up to 3 months.

Component	Cat. No.	Qty
ExMPro Reagent A	CC6204	0.51 g × 4
Regent A Diluent	CC6208	5 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 Plus Kit is optimized for immunofluorescence staining of cultured cells and enables uniform three-dimensional expansion of approximately 8× , resulting in an effective increase in spatial resolution (from ~250 nm to ~30 nm with wide-field microscopy, and from ~120 nm to ~15 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 techniques.

Pre-Experiment Guidelines

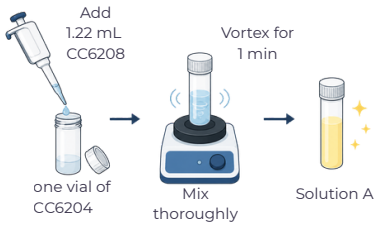
- **Applicable cell type:** animal cells
  - Adherent cells: ≥1 × 10^5 cells per sample
  - Suspension cells: >1 × 10^5 cells per sample
- **Not applicable to:** plant cells or cells with cell walls
- **Antibody optimization** (relative to standard dilution conditions):
  - Fluorescent secondary antibodies: from standard 1:1000 → ~1:100–1:200
  - Primary antibodies: from standard 1:500 → ~1:50–1:100
- **Signal requirement before expansion:**
  - Before proceeding with the expansion protocol, ensure that the fluorescence signal is bright and near-saturated under maximum excitation.
- **Additional Material Required:**
  - DAPI solution (1 µg/mL prepared in ddH_2O)
  - Cell Culture Coverslips
  - Dark Chamber
  - 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. Gel Preparation:

1.1



- **Add 1.22 mL CC6208 to one vail of CC6204**
- **Completely dissolved**

Pipette 1.22 mL of **Reagent A Diluent (CC6208)** into one vail of **ExMPro Reagent A (CC6204)**. Vortex thoroughly for approximately **1 minute** until completely dissolved and the solution becomes clear. A slight yellow coloration is acceptable.
Label the prepared **Solution A**, aliquot if needed, and store at −20°C. The solution will remain liquid at this temperature.
Then, prepare the gel solution according to Table 1.

1.2

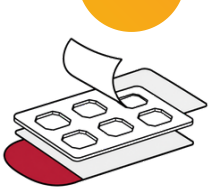
Mix gel solution

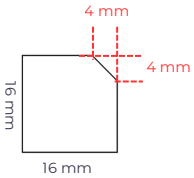
Table 1. Reagent volumes for gel solution (per sample)

Reagents	Volume
Solution 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





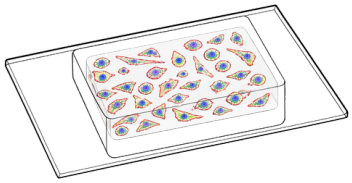
- **Add 300 µL of gel buffer per well**
- **Incubate in the dark for 45 minutes**

2. Gel Embedding:

Peel off the sticker and firmly attach the adhesive side to the bottom of the casting frame. Place the 16 × 16 × 1 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 a new adhesive sticker onto the casting frame. Incubate in **the dark for 45 minutes.**

3

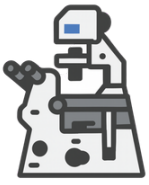


Expand 4 °C overnight

3. Expansion:

Remove the stickers. Place the gel containing the cell culture coverslips into excess water. Allow the gel to expand **in the dark at 4 °C overnight**.

4



Imaged directly with an inverted fluorescence microscope

4. Imaging:

Discard the excess water and carefully remove the expanded gel. Re-stain 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 the expanded gel into manageable sections, and transfer to a glass-bottom culture dish. Add a small amount of ddH_2O to keep the gel hydrated and 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**.

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 expansion, DAPI dissociates from nucleic acids and therefore requires re-staining to facilitate sample localization and rapid focusing. Protect the sample from light during the staining procedure. Do not prepare the DAPI solution in PBS, as this may cause gel shrinkage.
4. To achieve a linear gel expansion factor between 3.5× and 5.5×, expansion, use PBS at the dilution factors indicated below (prepared by diluting 1× PBS with ddH_2O). Refer to the table below:

Expansion Buffer	0.5 × PBS	0.3 × PBS	0.1 × PBS	ddH_2O
Gel Expansion Factor	3.5×	4.5×	5.5×	8~10×

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