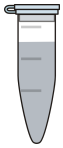
Expansion Microscopy Kit

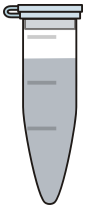
DIRECT PROTEIN ANCHORING VERSION FOR CULTURED CELLS

INSTRUCTION MANUAL

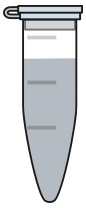
CONTENTS



Initiator
x4 (20 mg)



Accelerator
(20µL)

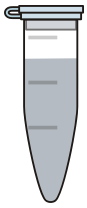


Digestion enzyme
(100µL)

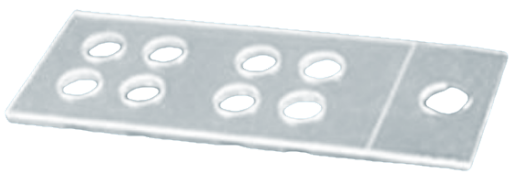
Anchoring
reagent
(5 µL)

Digestion
buffer
(10 mL)

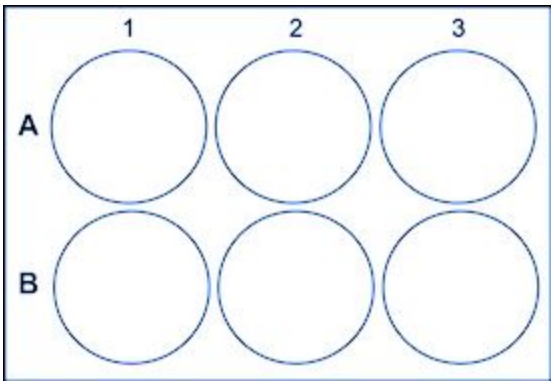
Non-adhesive glass slide **x 1**



monomer solution
(800 µL)



8 well chambered coverglass
for cell culture **x 4**



6 well imaging plate **x 1**

Storage

Component Name	Optimal Storage Temperature
Digestion Enzyme	-20 ^{0}C
Digestion Buffer	-20 ^{0}C
Monomer Solution	-20 ^{0}C
Accelerator	Room Temperature
Initiator	Room Temperature
Anchoring Reagent	-20 ^{0}C

Prior to use:

Dissolve contents of Initiator in 200 µL of DI water immediately before polymerization step.

Thaw all solutions stored at -20 ^{0}C (see table above), and ensure that no precipitate forms before using. If precipitate forms, vortex the vials briefly to redissolve compounds.

Preparation suggestions

Seed 1.5k - 3k cells with 60 µL of Media per well, using the included 8 well chambered coverglass (This will be used as the gelation and digestion chamber). Upon seeding the cells in 8 well chambered cover glass, place it into a 100 mm sterile dish (not included) and leave until the digestion step, to prevent evaporation of liquids such as media and anchoring solution.

Fix and Permeabilize the Cells using a standard immunostaining protocol. We recommend fixation with paraformaldehyde (PFA) solution or PFA/Glutaraldehyde (not included), and permeabilization with 0.1% Triton X and 1X PBS for 15 minutes at RT. Wash the cells with 1X PBS and use a standard immunocytochemical procedure to block and stain the cells with primary and secondary antibodies.

For overnight incubations, add 2-4 mL to the sides of the sterile dish to prevent evaporation of solutions in the wells.

Cover the dish with aluminium foil to protect the fluorophores bleaching from light

Instructions

Note: The Xpandit Antibody-Free Kit is good for 4 experiments and meant for use after **Secondary Antibody Staining**.

Note: **Expansion Microscopy causes a reduction in fluorescence** (specifically with Cy3, Cy5, and Alexa 647 dyes therefore Secondary Antibodies with these dyes are not recommended). We recommend Secondary Antibodies with Alexa 488, Atto 488, Alexa 594, Atto 647N, CF633, CF405M dyes.

We highly recommend performing a round of sample imaging after secondary antibody staining and **before** initiating sample anchoring.

We recommend using the included 8 well chambered coverglass for culturing, fixation, and staining

SAMPLE ANCHORING

S1. Dilute the **Anchoring Reagent** 1:100 in 1X PBS and add 60 µL to each well with cultured cells.

S2. Incubate at least 6 hours to overnight (12 to 16 hours) at room temperature. [*Note*:To prevent evaporation of anchor solution for overnight incubations, add 2-4 mL of water to the sides of the sterile dish.]

S3. Wash with 1X PBS for 5 minutes

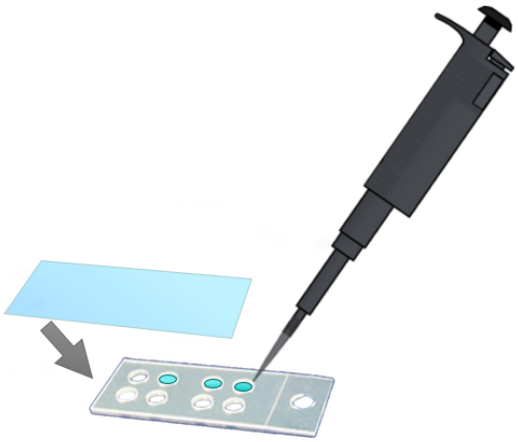
S4. Repeat step **S3** three times

POLYMERIZATION

P1. Dilute one of the **Initiator** tubes with 200 µL of DI purified water. Ensure that **Monomer Solution** has been vortexed such that no precipitate forms and solution is not cloudy.

P2. Prepare a polymerizing solution by adding 192 µL of **Monomer Solution** to a new vial followed by 4 µL of **Accelerator** and 4 µL of **Initiator**. Vortex and add 34 µL of the polymerizing solution to each well.

P3. Working quickly, pipette up and down briefly and ensure no air pockets form in the well. **CAUTION: Proceed quickly with this step as polymerization has started.**



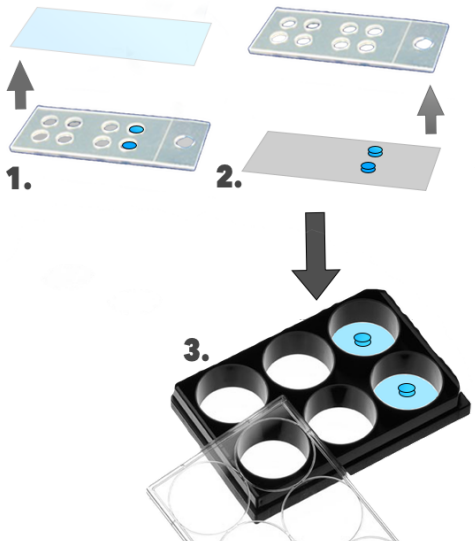
P4. Place the **Non-Adhesive Glass Slide** over the top of the gasket to seal the reaction vessel. **[Note: Place the smooth surface of the slide facing down]**

P5. Allow the samples to sit for 30 min at room temperature out of light to ensure proper polymerization.

The redissolved **Initiator** may be discarded after each polymerization is complete.

DIGESTION AND EXPANSION

D1. Prepare digestion solution by mixing 25 µL of **Digestion Enzyme** and 2475 µL of **Digestion Buffer** to a new vial.



D2. Carefully remove **Non-Adhesive Glass Slide**, peel the silicone gasket and carefully slide each gel into the bottom of the included 6 well plate and add total of 2.5 mL of the **Digestion Buffer** and **Digestion Enzyme** solution from the new vial to each well.

D3. Leave the plate on a rocker overnight at room temperature.

D4. Remove the **Digestion Buffer** and **Digestion Enzyme** solution, then wash the gels with at least 10 mL of ultrapure (cell culture grade) water for 30 min .

D5. Replace the water and repeat **three times**.

Within one half hours, Expansion will reach an equilibrium of 4.0X — 4.2X after 3 washes and fluorescence microscopy can be used to image the expanded samples in the 6 well imaging plate.

ADDITIONAL INFORMATION

The quality of water is **extremely** important. Water of lower purity will result in a lower total expansion. We recommend to use cell culture grade water

Imaging recommendation: For short term imaging, we recommend removing the water from the Expansion Plate so that the gel remains immobile in the plate. For long term imaging, the gel can be embedded in agarose.

Troubleshooting

The gel is moving during imaging causing difficulties during image capture
Ensure that all water has been removed from the wells before imaging. If the gel continues to move, use 0.5% agarose to embed the gel within the well plate.

The signal-to-noise ratio is very low during imaging/ the sample is very dim
Exposure of the sample to free radical polymerization (P5) and expansion (D5) causes reduction in overall sample brightness. Due to variation in the extent of fluorescence reduction, we recommend **not** using Cy3, Cy5, and Alexa 647 fluorophores.

The expansion factor of the gel is below 4x.
The sodium polyacrylate gel must be expanded with ultrapure water (such as cell culture water). Do two more extra cycles of washing if you don't see 4x expansion

DAPI Stain
If you have to stain with DAPI, we recommend to do it during the 2nd round of water wash during the expansion