Protocol for Expansion microscopy on cultured cells.

Based on Asano et al. 2018 Current Protocols in Cell Biology (read for more details)

Stock solutions are made as described in Asano et al. and are available from the imaging facility. Contact Lisbeth for more details.

Cell culture

Culture cells on PDL coated 13 mm coverslips.

Fix in 4 % PFA 10 min (PFA has to be fresh).

Quench the PFA with a 5 min wash step in 100 mM glycine in 1xPBS. Wash x 2 in 1xPBS

Can be stored at this stage @4C. However, for first test it is recommend to proceed to staining immediately.

Immunostaining - follow protocol for your antibody.

To obtain good staining after expansion it may be necessary to increase the concentration of the primary antibody compared to what is normally used.

OBS note on use of secondary antibodies. Cy3, Cy5 and Alexa 647 are degraded during the polymerization reaction. For farred dyes Atto 647 N or CF 633 is recommended. Also note that even though GFP is relatively resistant to Proteinase K treatment approx. 50 % of the signal may be lost. Other fluorescent proteins may be less resistant to proteinase K treatment. Secondary antibodies labeled with Alexa 488 and Alexa 568 are tested and found to work with this protocol.

Gelation

- Replace sample buffer with 0.1 mg/mL AcX solution (1:100 dilution of stock in 1xPBS) and incubate for 2 to 3 hours at RT (alternative 6h to ON) – no shaking (Have used ON incubation)
- During incubation make the gelation chamber (see *Asano et al. 2018)*
- Wash 2x 15 min in 1xPBS
- Make up gelling solution by mixing Stock X, water, TEMED and APS stock solutions in a 47:1:1:1 ratio (keep the gelling solution at 4 C until use) (need 200 µL for a gelling chamber of 22x22x0,38 mm) (OBS for 18 mm coverslips 350µl is needed)
- After washing in 1xPBS transfer coverslips with cells to the gelling chamber add gelling solution and put the top coverslip on. Make sure that the “chamber” is full of gelling solution and contains no air bubbles.
- Place the gelation chamber in an incubator at 37 C for 1 h for polymerization.

Digestion

- Take out the gelation chamber from the incubator.
- Use a razor blade to remove the top coverslip and remove the spacers.
- Take a picture of the gel to document the size before expansion.
- Transfer to 6 well plates.
- Add protein K solution 8U/mL diluted in digestion buffer (1mL pr well)

OBS the gel will expand slightly during the digestion.

Expansion

Remove digestion buffer and add 1xPBS (The gel can be stored at this stage – in the dark)

Add water to the petri dish and wait for 20 min. Do 2 additional water changes, 20 min each.

Take picture of expanded gel

Cut the gel into appropriate sizes for imaging.

Gently move the gel to a glass bottom dish or 6 cm plate for imaging (depending on whether the sample is to be imaged on an inverted or upright microscope). Coating the imaging dish with PDL helps to keep the gel attached to the surface. Make sure that the gel is orientated right for the type of microscope used. Keep the gel hydrated.

Image right after expansion. Signal is lost quite fast.

Reagents for expansion microscopy

Available at imaging facility

Anchoring

AcX Thermo fisher cat. No. A20770 Stock solution at 10 mg/mL in DMSO

AcX stock solution stored at -20 C in 20 µl aliquots (to be used at 1:100 in 1x PBS)

Gelation

Stock X -solution is stored at -20 C in 1 mL aliquots. (see content in Asano et al.)

N,N,N,N-tetramethylethane-1,2-diamine (TEMED), Thermo Fischer no 17919 (469,-) stock at 10 g/ 100 mL. Stored at -20 C in 500 µl aliquots.

APS Thermo fisher Cat. No 17874. Stock solution 10 g/ 100 mL. Stored at -20 C in 500 µl aliquots.

Stop reagent

4-hydroxy-TEMPO (4-HT) (Alfa Aeser A12497): 5 mg/mL stock in ultrapure water. Store at −20 °C. The stock solution can be stored for several months.

Digestion

Proteinase K (have stock at 20 mg/ml Ambion AM2548 @ 600 u/mL, check activity for each batch this may change)

Digestion buffer 1 stored in 1 ml aliquots at -20˚C. (see content in Asano et al. 2018)

Stock solutions to prepare.

4% PFA in 1xPBS for fixation

1xPBS

Reagents for immunostaining