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# Immunofluorescence under confinement

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**We use this protocol and it's working**

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## Abstract

Cells under agarose confinement were fixed and labelled with desired antibodies.

## Immunofluorescence under confinement

5h 20m

1 Samples under agarose pad confinement were fixed with 4% paraformaldehyde (PFA; Electron Microscopy Sciences, 15710) in cytoskeleton buffer (CB; 10 mM MES, 3 mM $MgCl_2$, 138 mM KCl, 2 mM EGTA) for 20 min at 37 °C, after which the agarose pad was gently removed. 20m

2 Cells were permeabilized with 0.5% Triton X-100 in CB for 5 min at room temperature (RT) and quenched with 10 mM glycine in CB for 5 min. 10m

3 Samples were washed with Tris-buffered saline (TBS; 20 mM Tris, pH 7.6, 137 mM NaCl) (2 × 5 min, then 2 × 10 min) and blocked for 1 h at RT in blocking buffer (2% IgG- and protease-free BSA and 0.1% Tween-20 in TBS). 1h 30m

4 Cells were incubated for 2h at RT or overnight at 4 °C with primary antibodies against CD44 (BJ18;
BioLegend), Ezrin (3C12; Invitrogen), EGFR (H11; Invitrogen), or phospho-ERM (Thr567/564/558; Cell Signaling Technology) diluted in TBS. 2h

5 Following three TBS washes, samples were incubated for 1 h at RT with fluorophore-conjugated secondary antibodies (1:500; Jackson ImmunoResearch) and Alexa Fluor 647–phalloidin (1:200; Thermo Fisher Scientific) diluted in blocking buffer. 1h

6 Samples were washed with TBS (2 × 10 min) and mounted in Dako mounting medium (S3023) on glass-bottom dishes. 20m