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dSTORM of actin in fixed HeLa cells

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Protocol status: Working

We use this protocol and it's working



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Abstract

This is a protocol for the preparation of a HeLa DTS cells for dSTORM imaging of the actin network. This protocol was used to generate the data shown in **Figure 4** of the following publication:

- Bruggeman et al., POLCAM: Instant molecular orientation microscopy for the life sciences. bioRxiv 2023.02.07.527479 (Feb 2023), doi: <https://doi.org/10.1101/2023.02.07.527479>



Cell culture

- 1 HeLa TDS cells were cultured in DMEM (Gibco, Invitrogen) supplemented with 10 % Fetal Bovine Serum (FBS, Life Technologies), 1 % penicillin/streptomycin (Life Technologies), and 1 % glutamine (Life Technologies) at 37 °C + 5 % CO₂.
- 2 Cells were periodically tested for mycoplasma contamination and passaged 3 times per week.
- 3 Cells were plated at low density on high-precision glass coverslips (MatTek, P35G-0.170-14-C) 1 day prior to fixation for dSTORM experiments.

Fluorescent labelling

1h 41m

- 4 Simultaneously fix and permeabilize the cells in cytoskeleton buffer (CBS, 10 mM MES, 138 mM KCl, 3 mM MgCl₂, 2 mM EGTA, 4.5 % sucrose w/v, pH 7.4) + 4 % paraformaldehyde (PFA) and 0.2 % Triton for 00:06:00 at 37 °C
- 5 Further fix the cells in CBS + 4 % PFA for 14 Mass Percent at 37 °C .
- 6 Wash the cells 3 times in PBST (PBS supplemented with 0.1 % Tween).
- 7 Permeabilize a second time in PBS + 0.5 % Triton for 00:05:00 at Room temperature .
- 8 Wash the cells 3 times in PBST.
- 9 Blocked the cells for 00:30:00 in 5 % BSA.
- 10 Wash the cells 3 times in PBST.

6m

5m

30m



- 11 Incubate the cells with Alexa FluorTM 488 Phalloidin (A12379, Invitrogen, 1:50 in PBS) for  01:00:00 in the dark.
- 12 Wash the cells 3 times in PBS.
- 13 Prior to dSTORM imaging, replace the PBS with dSTORM imaging buffer (base buffer consisting of 0.56 M glucose, 50 mM Tris (pH 8.5), and 10 mM NaCl supplemented with 5 U/mL pyranose oxidase (Sigma, P4234), 10 mM cysteamine (Sigma, 30070), 40 µg/mL catalase (Sigma, C100) and 2 mM cyclooctatetraene (Sigma, 138924).

1h