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## Live-cell imaging of the plasma membrane of Jurkat T cells

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

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## Abstract

This is a protocol for the preparation of a Jurkat T cells for live imaging of the plasma membrane. This protocol was used to generate the data shown in **Figure 6** 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 J8 LFA-1 cells were incubated overnight (approximately  18:00:00 ) in complete-RPMI (StableCell RPMI-1640 media, Sigma) supplemented with 10 % (v/v) fetal calf serum (FCS), 1 % (v/v) HEPES buffer, and 1 % (v/v) pen/strep antibiotics. 18h
- 2  1 mL of cells were collected by centrifugation and resuspended in phenol-red free RPMI supplemented with 1 % HEPES.

## Cell Labeling

50m

- 3 Round coverslips were rinsed with IPA, MilliQ, dried, and Ar-plasma cleaned for  00:20:00 . 20m
- 4 Grace Bio-Labs CultureWells were attached, and the slide was incubated with OKT3 antibody (provided by the Human Immunology Unit, WIMM, Oxford) for  00:30:00 . 30m
- 5 The slide was washed 5 times with phenol-red free RPMI supplemented with 1 % HEPES.
- 6 Perform a final wash with phenol-red free RPMI supplemented with 1 % HEPES and 200 nM NR4A (MemGlow™ NR4A Membrane Polarity Probe, Cat. #MG06, Cytoskeleton Inc.) before imaging.