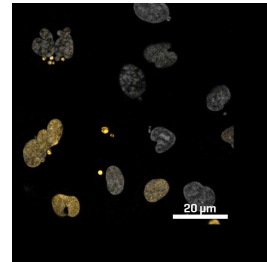


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m6A visualization/immunofluorescence of DamID

DOI

<https://dx.doi.org/10.17504/protocols.io.e6nvwdz6zlmk/v1>



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Protocol status: Working

We use this protocol and it's working

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Abstract

Used for visualizing Dam activity by immunofluorescence in mammalian cells. This protocol stains N-6 methyladenosine (m6A) modified DNA by immunofluorescence while preserving other epitopes, avoiding harsh denaturation steps like heat or chemical treatments.

Materials

100% MeOH

PBS

BSA

TritonX-100

Sodium azide

DpnI (NEB #r0176)

CutSmart buffer (NEB #b7204)

Rabbit anti-m6A (Synaptic #202 003)

Secondary antibody



Protocol

- 1 Fix cells in 100% MeOH for 10 min at -20 °C
- 2 Block, and RNase treat in 2 ug/mL RNase A in blocking buffer (3% BSA, 0.04% TritonX-100, 0.02% sodium azide in PBS) at 37 °C for 30 min
- 3 Wash cells 2x in PBS for 5 min at room temperature
- 4 Permeabilize cells in 2% TritonX-100 in PBS for 10 min at room temperature
- 5 Digest in 50 U/mL DpnI (NEB #r0176) in 1x CutSmart buffer for 30 min at 37 °C
- 6 Wash 3x in PBS for 5 min at room temperature
- 7 Incubate cells in rabbit-anti-m6A (Synaptic, #202 003) diluted 1:500 in PBS for 30 min at room temperature in a humidified chamber
- 8 Wash 3x in PBS for 5 min at room temperature
- 9 Incubate cells in anti-rabbit, AlexaFluor conjugated antibody in blocking buffer, diluted according to manufacturer's suggestion, for 30 min at room temperature in a humidified chamber in the dark
- 10 Proceed with further immunofluorescence steps and/or DNA staining and mount cells