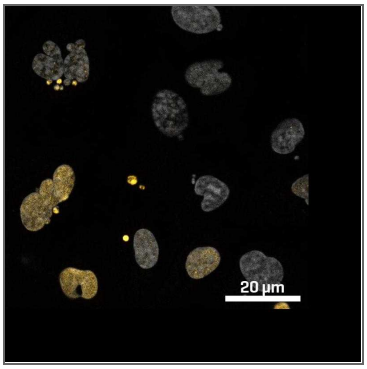

OCT 03, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.e6nvwdz6zlmk/v1

Protocol Citation: Lucian Dipeso, Emily Hatch 2023. m6A visualization/immunofluorescence of DamID. **protocols.io** https://dx.doi.org/10.17504/protocols.io.e6nvwdz6zlmk/v1

Protocol status: Working
We use this protocol and it's working

Created: Oct 03, 2023

m6A visualization/immunofluorescence of DamID

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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

Last Modified: Oct 03, 2023

PROTOCOL integer ID:
88738

Keywords: DamID, m6A,
Immunofluorescence

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