Oct 27, 2020

3D Immunostaining for CLARITY-processed samples

DOI
https://dx.doi.org/10.17504/protocols.io.bnzdmf26

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Protocol Citation: Seth Currlin 2020. 3D Immunostaining for CLARITY-processed samples. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bnzdmf26

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

Created: October 27, 2020

Last Modified: October 27, 2020

Protocol Integer ID: 43781

Keywords: 3d immunostaining for clarity, 3d immunostaining, immunostaining large sample, immunostaining clarity, human thymus tissue, piece of human thymus tissue, large tissue volume, particular tissue, dense tissue type, processed sample, mm3 in size, large sample

Abstract

This is a guide for immunostaining CLARITY-processed samples. **dx.doi.org/10.17504/protocols.io.8jihuke**

These steps are meant to be a guide for immunostaining large samples and should be optimized to suit your particular tissues and reagents. Large tissue volumes and dense tissue types will require longer incubation and wash times. The parameters suggested below are for a piece of human thymus tissue approximately 5 mm^3 in size.

A useful link: http://wiki.claritytechniques.org/index.php/Immunostaining

Materials

Solutions:

Wash buffer (PBST): 1x phosphate buffered saline (PBS), 0.5% Triton X-100, pH 7.4; at least 20x volume of tissue.

Blocking buffer: 1x PBS, 1% horse serum, 0.5% Triton-X 100, 0.05% Tween-20, pH 7.4; at least 10x volume of tissue.
oAnother serum type should be used based on the secondary antibody.

Staining buffer: 1x PBS, 0.5% Triton X-100, 0.05% Tween-20, pH 7.4; at least 10x volume of tissue.

Before start

Ensure the tissue is sufficiently transparent for your imaging requirements. If the sample has not been successfully cleared then subsequent imaging will be difficult due to regions retaining light-obscuring lipids. Samples should be imaged prior to immunostaining to confirm adequate clearing, particularly among excitation/emission settings intended for later use.

Immunohistochemistry for large (5 mm^3) tissue volumes

3w 5d

1 Wash out residual clearing solution — 3d

Three washes in PBST, 24 hours each, 37C with gentle agitation.

2 Nonspecific Epitope Blocking — 3d

Blocking buffer incubation: three days, 37C with gentle agitation.

Note: Serum type should be used based on the secondary antibody host species.

3 Primary Antibody Incubation — 1w

1:100 dilution (~10 ug/mL) in Staining Buffer.

Primary antibody incubation: five days, 37C with gentle agitation; then two days, 4C with gentle agitation.

4 Wash out Primary Antibody — 3d

Three washes, 24 hours each, at 37C with gentle agitation.

5 Secondary Antibody Incubation — 1w

1:200 dilution (~5 ug/mL) in Staining Buffer.

Secondary antibody incubation: five days at 37C with gentle agitation; two days at 4C with gentle agitation.

Note: Protect samples from light.

6 Wash out Secondary Antibody — 3d

Three washes, 24 hours each, at 37C with gentle agitation.

Note: Protect samples from light.

7 Prepare for 3D Imaging or Storage

Prepare for imaging or store samples in washing buffer (PBST) at 4C.

For 3D imaging guide, see: https://dx.doi.org/10.17504/protocols.io.begajbse