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 We use this protocol and it's working

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🌐 Large Volume Immunostaining for Cleared Samples

Seth Currlin¹, Marda Jorgensen¹, Jerelyn Nick¹

¹University of Florida

Optical Clearing of Tissue

Human BioMolecular Atlas Program (HuBMAP) Method Development Core

Seth Currlin

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 tissue (thymus, spleen, or lymph node) approximately 5 mm³ 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 INSTRUCTIONS

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 mm³) tissue volumes

3w 5d

1 Wash out residual clearing solution

3d

Three washes in PBST, 24 hours each, 37°C with gentle agitation. Large wash volumes are best, a 50 ml conical tube with 50 ml fresh PBST is recommended for each wash step.

2 Nonspecific Epitope Blocking

3d

Blocking buffer incubation: three days, 37°C with gentle agitation. Blocking buffer volume should be at least 5 volumes greater than tissue volume.

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, 37°C with gentle agitation; then two days, 4°C with gentle agitation.

For best results a large volume for primary antibody incubation is recommended however. A 5 ml total volume for antibody staining is recommended for a 5 mm³ piece of tissue.

4 Wash out Primary Antibody

3d

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

5 Secondary Antibody Incubation

1w

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

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

Note: Protect samples from light.

6 Wash out Secondary Antibody

3d

Three washes, 24 hours each, at 37°C 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 4°C.

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