CLARITY Tissue Immunostaining

Product Overview

CLARITY is a tissue clearing method used to render tissues optically transparent, allowing for non-destructive tissue processing and 3D imaging of both normal and diseased soft tissues. In the CLARITY tissue clearing method, the tissue is first embedded with hydrogel monomers to create a matrix scaffold, then the light scattering lipids are removed. After lipid clearing, the tissue-hydrogel hybrid is both transparent to light and permeable to molecules, enabling immunostaining and imaging using a confocal or light-sheet microscope.

This kit is intended for Research Use Only.

Kit Contents

The CLARITY Tissue Immunostaining component:

- Immunostaining Buffer (not included in CLARITY Tissue Clearing Kit) (105 mL) – Store at room temperature

Please inspect the contents upon receipt to confirm all parts have been included and ensure that no damage occurred during shipment. Check that all containers are sealed and that none of the seals have been broken. Any damage occurring as a result of shipping and handling should be filed with the carrier.

Safety Warning and Precautions

All reagents should be considered hazardous. Appropriate personal protective equipment (PPE, i.e., lab coat, gloves, goggles) should always be worn when using this kit.

CLARITY immunostaining should only be performed by personnel trained with good laboratory practices and techniques.

Please review the Safety Data Sheet(s) for safe handling, disposal, and storage conditions for all reagents listed herein prior to use.

Procedural Guidelines

- This reagent has been optimized for the immunostaining of soft tissues cleared using the *CLARITY Tissue Clearing Kit*.
- Perform all steps at room temperature (20-25°C) unless otherwise noted.
- Wear disposable gloves while handling the reagents and samples.
- Use disposable, sterile plasticware, pipette tips, and tubes.
- Samples with fluorescently labeled endogenous markers should be protected from light throughout the entire procedure.

Required Equipment and Materials (not supplied)

- ddH_2O
- Incubator/shaker
- Spatula(s)
- Washing buffer (Recommend: *Wash Buffer 2 from CLARITY Tissue Clearing Kit*)
- Pipettes and pipette tips (various sizes)
- Multi-well plates (various sizes)
- Plate sealing tape
- Blocking buffer, if preferred
- Antibodies of interest (primary and secondary), if necessary
- Nuclear counterstain, if necessary
- Refractive Index matching solution
- Imaging platform
    - Confocal or light-sheet microscope

Before you Begin – Preparatory Work

Use the tissue/sample(s) previously cleared with the *CLARITY Tissue Clearing Kit.*

Immunostaining Buffer is provided at a **10X concentration**. Dilute Immunostaining Buffer with 895 mL of ddH_2O to a total of 1000 mL solution before use.

Procedures

Immunostaining

1. Blocking samples: Block the sample(s) using your preferred blocking solution.
    - Blocking buffer should be prepared ahead of time.
    - Note: Not all sample types will require blocking, but we recommend this step to reduce non-specific binding.
2. In a new multi-well plate, use a spatula to place each washed lipid-cleared sample into one well in an appropriately sized multi-well plate (*i.e.*, 1 section per well).
    - Size-appropriate means that the volume of each well should be as small as possible, while still enabling the sample to lay flat. This allows use of smaller volumes of blocking and staining buffers.
3. Tissue sections should be fully covered by blocking buffer in each well.
    - Note: We recommend filling any empty wells remaining with H_2O to minimize evaporation.
4. Cover plate with sealing tape.
5. Place in a shaking incubator at 37°C for 18-24 hours.
6. Immunostaining samples: Prepare antibody dilutions using the Immunostaining Buffer.
    - We recommend starting at ~10 µg/mL, if possible, to test feasibility for a thick tissue section.
    - Previously tested antibodies should be used at previously assessed optimal dilutions/concentrations.

- Prepare enough antibody solution to fully cover each tissue sample in the well-plate. 200 µL is typically enough to cover a 1 mm thick sample in a well of a 48-well plate; however, more solution may be needed for thicker sample sections or wells of larger volumes.

7. Gently lift the sealing tape from the plate.
8. Carefully remove the blocking buffer solution from each sample well using a pipette.
9. Add the prepared antibody dilutions to each sample well until the sample is fully covered.
10. Reseal the plate with sealing tape.
    - We recommend that all remaining empty wells be filled with H_2O to minimize evaporation.
    - Note: **If using directly conjugated Ab°s**, protect the plate from light exposure to preserve the fluorophores.
11. Place sealed plate into a shaking incubator for 1-7 days at 37°C.
    - The duration of the immunostaining process is dependent upon the thickness of the sample.
    - Recommendation based on sample section thickness:
        - <1 mm: 1-2 days
        - 1-2 mm: 3 days
        - 3-5 mm: 5 days
        - >5 mm: 1 week
12. Carefully remove primary Ab° solution from the sample well(s).
13. Wash tissue section(s) with your preferred washing solution by adding the wash buffer to each sample and gently shaking for 24 hours.

- We recommend using Wash Buffer 2 (WB2), and exchanging the wash buffer multiple times.

14. **<u>Indirect secondary Ab° conjugation only</u>**: Prepare secondary Ab°(s) and nuclear counterstain dilutions in Immunostaining Buffer.
    - We recommend an initial secondary Ab° concentration of ~10 µg/mL.
    - Prepare enough Ab° and counterstain solution to fully cover each tissue sample in the well-plate.
15. Add the secondary Ab° and counterstain solutions to each sample well so that it covers the entire sample.
16. Reseal the plate with the sealing tape.
    - We recommend that all remaining empty wells be filled with H_2O to minimize evaporation.
    - <u>Note</u>: Protect the plate from light exposure to preserve the fluorophores.
17. Place the sealed plate into a shaking incubator for 1-7 days at 37°C.
    - The duration of the immunostaining process is dependent upon the thickness of the sample.
    - Recommendation based on sample section thickness:
        - <1 mm: 1-2 days
        - 1-2 mm: 3 days
        - 3-5 mm: 5 days
        - >5 mm: 1 week
18. Carefully remove secondary Ab° solutions from each sample well using a pipette.

19. Wash tissue sections with preferred washing solution by adding wash buffer to each sample and gently shaking for 24 hours.
    - We recommend using WB2 and exchanging the wash buffer multiple times.

Proceed to your preferred RI matching solution and imaging protocol for the samples.

References:

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Sylwestrak, E. L. *et al*. Multiplexed Intact-Tissue Transcriptional Analysis at Cellular Resolution. *Cell* **164**, 792-804, 2016.

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