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1. Description

This product is for research use only.

Components	100 mL Permeabilization Solution 125 mL 10× Antibody Staining Solution 100 mL Clearing Solution
Specifications	The refractive index of the Clearing Solution is 1.556–1.562.
Storage	Upon arrival store all components at +2 to +8 °C protected from light. Permeabilization Solution and 10× Antibody Staining Solution are supplied in buffer containing stabilizer and 0.09% sodium azide. Permeabilization Solution is ready-to-use. For detailed information about usage and storage of the different components refer to chapter 2. The expiration date is indicated on the vial label.

1.1 Principle of the MACS Clearing Kit

The MACS Clearing Kit enables the immunostaining and clearing of different tissues for 3D imaging analysis. The kit has been developed for the easy, fast, and effective immunostaining and clearing of different tissues, such as mouse brain hemispheres, cerebral organoids, human primary tumors, or patient-derived xenografts. Different tissue-specific protocols have been developed to ensure the efficient immunostaining and clearing of each tissue type for optimal imaging results.

1.2 Background information

Fluorescence imaging of large biological systems in three dimensions (3D) requires working with optically transparent tissues. However, most tissues are opaque, which results in low imaging depth. Therefore, tissues need to be treated with chemical components in order to render them transparent; a process also known as tissue clearing.

Cleared tissues can then be imaged using different microscopy systems to study their internal morphology or reveal the presence of certain cell populations or cellular structures, such as the tissue vasculature.

1.3 Applications

- Immunostaining and clearing of different complex biological structures, such as mouse brain, cerebral organoids, or tumor samples for 3D imaging analysis thereof using light sheet fluorescence microscopy, confocal, or two-photon microscopy.

1.4 Reagent and instrument requirements

- Sterile distilled water
- MACSmix™ Tube Rotator (# 130-090-753)
- MACS Imaging Solution (# 130-126-335)

2. Protocol

▲ Clearing Solution is a non-toxic organic solvent. It might not be compatible with all plastics. Use polypropylene tubes or containers.

▲ Clearing Solution is ready-to-use but may freeze at +2 to +8 °C. Therefore, it is recommended to prepare aliquots of appropriate volume and store at +2 to +8 °C. Adjust aliquots to room temperature before proceeding to clearing of tissues.

▲ 10× Antibody Staining Solution needs to be diluted 1:10 with sterile distilled water before use. Prepare 1× Antibody Staining Solution by diluting 1 mL of 10× Antibody Staining Solution with 9 mL of sterile distilled water. Adjust the volume of 1× Antibody Staining Solution to the amount of tissue to be cleared. Always use freshly prepared 1× Antibody Staining Solution.

▲ **Note:** Before preparing the dilution, make sure that the 10× Antibody Staining Solution does not contain any precipitates.

▲ For more information about protocols to clear different tissues refer to www.miltenyibiotec.com/tissue-clearing-protocols.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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