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### 1. Description

This product is for research use only.

<b>Components</b>	100 mL Deep Permeabilization Solution 87.5 mL Deep Antibody Staining Solution (8×) 100 mL Deep Clearing Solution
<b>Specifications</b>	The refractive index of the Deep Clearing Solution is 1.556–1.562.
<b>Storage</b>	Upon arrival store all components at +2 to +8 °C protected from light. Deep Permeabilization Solution and Deep Antibody Staining Solution (8×) are supplied in buffer containing stabilizer and 0.09% sodium azide. Deep 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 Deep Clearing Kit

The MACS Deep Clearing Kit enables the easy, fast, and effective immunostaining and clearing of mouse whole brains or brain hemispheres for 3D imaging analysis. A protocol specific for clearing of mouse brains has been developed to ensure the efficient immunostaining and clearing of samples 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 mouse whole brain or brain hemispheres for 3D imaging using light sheet fluorescence microscopy, confocal, or two-photon microscopy

### 1.4 Reagent and instrument requirements

- 1× PBS, pH 7.4
- MACSmix™ Tube Rotator (# 130-090-753)
- MACS Imaging Solution (# 130-126-335)

### 2. Protocol

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

▲ Deep 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.

▲ Deep Antibody Staining Solution (8×) needs to be diluted 1:8 with 1× PBS, pH 7.4 before use. Prepare Deep Antibody Staining Solution (1×) by diluting 1 mL of Deep Antibody Staining Solution (8×) with 7 mL of 1× PBS, pH 7.4. Adjust the volume of Deep Antibody Staining Solution (1×) to the amount of tissue to be cleared. Always use freshly prepared Deep Antibody Staining Solution (1×).

▲ **Note:** Before preparing the dilution, make sure that the Deep Antibody Staining Solution (8×) does not contain any precipitates.

▲ For more information about protocols to clear different tissues refer to [www.miltenyibiotec.com/tissue-clearing-protocols](http://www.miltenyibiotec.com/tissue-clearing-protocols).

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