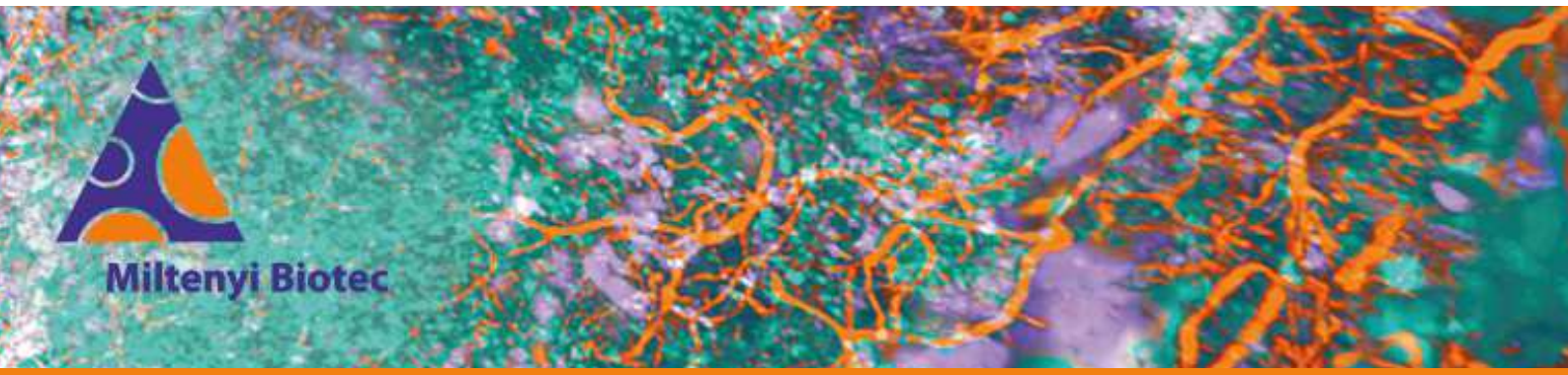

MACSima™ Imaging Platform

DAPI Pre-Staining Protocol

In this handout the DAPI prestaining protocol using Miltenyi's DAPI Staining Solution is either available alone or included in the MACSima™ Stain Support Kit. Pre-staining is required for the 2× overview scan, so you are able to draw regions of interest (ROIs) on your slide and identify interesting regions already by the nuclear stain.

Important: To perform the DAPI pre-stain, *your sample has to be fixed already*.

Fresh frozen samples: DAPI pre-staining protocol

Prerequisite: Fixed sample slices on a microscope slide already fixed in the MACSwell™ Imaging Frame of choice.

1. Prepare and add the DAPI Staining Solution by diluting it 1:5 in MACSima™ Running Buffer.
   Note: This can be done either separately in a reaction tube of choice or in the Imaging Frame cavity directly.

Table 1: Overview of DAPI and Running Buffer volumes per well depending on MACSwell™ format.

Disposable	Volume of DAPI/Well	Volume of MACSima Running Buffer/Well
MACSwell Four MACSwell 24	50 µL	200 µL
MACSwell Two MACSwell One Small	100 µL	400 µL
MACSwell One	200 µL	800 µL

2. Incubate for 10 minutes at room temperature in the dark.
3. Remove the supernatant, but be careful not to disturb the sample.
   Note: Try to pipet from the edges and tilt the frame to you can collect all the liquid.
4. Wash the well(s) 3× with MACSima Running Buffer, while making sure not to disturb the tissue section:

Table 2: Overview of washing volumes after DAPI staining depending on the MACSwell™ format.

Disposable	Washing volume per well
MACSwell Four MACSwell 24	475 µL
MACSwell Two MACSwell One Small	950 µL
MACSwell One	1,900 µL

5. Add the appropriate amount of initial sample volume of MACSima™ Running Buffer:

Table 3: Overview of initial sample volume depending on the MACSwell™ format.

Disposable	**Volume of MACSima Running Buffer/Well**
MACSwell Four MACSwell 24	475 µL
MACSwell Two MACSwell One Small	950 µL
MACSwell One	1,900 µL

6. Proceed with the antibody preparation and keep the stained sample at 4 °C, if not used directly.