Miltenyi Biotec

Fixation of exosomes on the MACSPlex Exosome Capture Beads

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1. Description

1.1 Background information

The MACSPlex Exosome Kit has been designed to determine surface marker profiles of extracellular vesicles (EVs). The analysis is based on MACSPlex Exosome Capture Beads, which display defined fluorescence properties and can be identified using standard flow cytometry techniques.
The following protocol has been developed to fix bound and stained vesicles on the MACSPlex Exosome Capture Beads to enable the analysis of potentially infectious material by flow cytometric analysis.

1.2 Reagent and instrument requirements

- MACSPlex Exosome Kit, human (# 130-108-813)
- Inside Stain Kit (# 130-090-477)
- MACSQuant® Analyzer or MACSQuant Analyzer 10 (# 130-096-343), or other flow cytometers equipped with blue (488 nm) and red (635 nm) lasers able to discriminate FITC, PE, and APC fluorescence.

  ▲ **Note:** The MACSQuant VYB cannot be used.
- MACS® Chill 96 Rack (# 130-094-459), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.
- MACSQuant Calibration Beads (# 130-093-607), when using the MACSQuant Analyzer or MACSQuant Analyzer 10.
- For microtiter plate format: orbital shaker for 96-well plates (450 rpm)
- For tube format: the MACSmix™ Tube Rotator (# 130-090-753) or an orbital shaker for tubes (450 rpm)
- (Optional) 1.5 mL reagent tubes
- (Optional) 96-well round bottom plate

2. Protocol

Follow the protocol of the MACSPlex Exosome Kit including the resuspension of the MACSPlex Exosome Capture Beads after the final washing step. Fix samples in the MACSPlex Filter Plate or 1.5 mL reagent tube as descibed below before flow cytometric analysis.

SP00980.01

2.1 Fixation using MACSPlex Filter Plates

1. Add 100 µL of Inside Fix per well.
2. Incubate for 20 minutes at room temperature protected from light on an orbital shaker (450 rpm).
3. Add 200 µL of the MACSPlex Buffer to each well.
4. Put the filter plate on a vacuum manifold and apply vacuum (max. –60 mbar) for about 30 seconds until wells are drained. Switch off the vacuum and ventilate the vacuum manifold.

   ▲ **Note:** Alternatively, centrifuge the filter plate at 300×g at room temperature for 3 minutes.
5. Add 150 µL of the MACSPlex Buffer to each well. Resuspend the MACSPlex Exosome Capture Beads carefully.
6. Proceed with flow cytometric analysis as described in the MACSPlex Exosome data sheet.

2.2 Fixation using 1.5 mL reagent tubes

1. Add 100 µL of Inside Fix to each reagent tube.
2. Incubate for 20 minutes at room temperature protected from light using a MACSmix Tube Rotator or an orbital shaker for tubes (450 rpm).
3. Add 1 mL of the MACSPlex Buffer to each reagent tube.
4. Centrifuge at 3000×g at room temperature for 5 minutes.
5. Remove 2×550 µL of the supernatant.
6. Resuspend MACSPlex Exosome Capture Beads carefully in remaining volume and transfer sample to a MACSPlex Filter Plate or a 96-well round bottom plate.
7. Proceed with flow cytometric analysis as described in the MACSPlex Exosome data sheet.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

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