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## 1. Description

**Components** 1 vial MACSxpress Erythrocyte Depletion Reagent, human – lyophilized: MACSxpress Beads conjugated to monoclonal antibodies.

2 mL MACSxpress Erythrocyte Depletion Buffer

**Capacity** For 100 mL of cell suspension isolated with a MACSxpress Isolation Kit.

**Storage** Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. For information about reconstitution of the lyophilized cocktail and storage after reconstitution refer to chapter 2.1.

### 1.1 Principle of the MACSxpress Erythrocyte Depletion Kit

The MACSxpress Erythrocyte Depletion Kit has been developed for the removal of residual erythrocytes after cell isolation using a MACSxpress Isolation Kit.

Residual erythrocytes after MACSxpress Isolation are magnetically labeled with the MACSxpress Erythrocyte Depletion Reagent. A tube containing the cell suspension is placed in the magnetic field of a MACSxpress Separator. The magnetically labeled erythrocytes will adhere to the wall of the tube. The isolated target cells remain unlabeled in the supernatant.

### 1.2 Applications

- Depletion of erythrocytes from cells isolated with a MACSxpress Isolation Kit.

### 1.3 Reagent and instrument requirements

- MACSxpress Separator
- 5 mL polystyrene round-bottom test tube or 15 mL or 50 mL tubes
- (Optional) MACSmix™ Tube Rotator (# 130-090-753)

## 2. Protocol

▲ Adjust all reagents and materials to room temperature (19–25 °C) before use.

▲ Pipette gently to avoid foam formation.

### 2.1 Reconstitution of MACSxpress Erythrocyte Depletion Reagent

Reconstitute the lyophilized MACSxpress Erythrocyte Depletion Reagent by adding the MACSxpress Erythrocyte Depletion Buffer (2 mL) to the vial. Mix by pipetting up and down until resuspended. After reconstitution the MACSxpress Erythrocyte Depletion Reagent is stable for 1 month at 2–8 °C. Write the new expiration date after reconstitution on the vial label.

### 2.2 Magnetic labeling

- Pipette cell suspension isolated with a MACSxpress Isolation Kit into an appropriate tube (refer to table below).

Sample volume	Tube size
1–4 mL	5 mL tube
5–12 mL	15 mL tube
13–20 mL	Split sample into several 15 mL tubes
21–45 mL	50 mL tube

- Add 20 µL of reconstituted MACSxpress Erythrocyte Depletion Reagent per 1 mL of cell suspension.
- Close the tube tightly and invert gently three times. Incubate sample for 5 minutes at room temperature using the MACSmix Tube Rotator on permanent run speed of approximately 12 rpm.  
▲ **Note:** If another rotator is used, make sure to adjust rotation speed.
- Proceed to magnetic separation (2.3).

### 2.3 Magnetic separation

1. Remove the tube containing the sample from the MACSmix Rotator and carefully open the cap.
2. Place the open tube in the magnetic field of the MACSxpress Separator for 10 minutes. The magnetically labeled erythrocyte will adhere to the wall of the tube.  
▲ **Note:** Do not move the tube during the separation process.
3. While the tube is still inside the MACSxpress Separator, carefully collect the supernatant into a new tube. The supernatant contains the target cell fraction.

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