CAR Dextramer® Staining Protocol

Products

CAR Dextramer®, CD19, human / PE, Cat. No. CT001 PE 50
CAR Dextramer®, BCMA, human / PE, Cat. No. CT003 PE 50
CAR Dextramer®, MSLN, human / PE, Cat. No. CT006 PE 50

Recommended use

CAR Dextramer® is recommended for use in flow cytometry for detection and monitoring of CAR cells.

Materials Required (not provided)

4 mL Falcon disposable 12 x 75-mm test tubes or equivalent
Stain and Wash buffer: PBS, 1-5% FCS, pH 7.4
Antibodies identifying relevant cell-surface markers and live-dead dye[A].

See the FAQ on immudex.com regarding recommended antibody clones. The optimal choice of fluorochromes depends on the flow cytometer and experimental setup.

Procedure

1. Prepare your PBMCs or CAR cells[B] by washing twice in 10 mL Stain and Wash buffer.
2. Resuspend 1-3 x 10^6 PBMCs or 1-2 x 10^5 transduced CAR cells in 50 µL Stain and Wash buffer
3. Centrifuge the CAR Dextramer® at 10.000 x g for 1 min. to avoid transferring any potential precipitate.
4. Add 10 µL of CAR Dextramer® reagent to the cell sample and vortex briefly[C].
5. Incubate in the dark at room temperature for 10 min. incubation.
6. Add relevant antibodies in the volume/concentration according to manufacturer's instructions[D].
7. Incubate at room temperature in the dark for 20 min.
8. Wash cells by adding 2 mL stain and wash buffer. Centrifuge at 300 x g for 5 min. and remove the supernatant. Repeat washing for a total of 2 washes[E].
9. Resuspend the pellet to the desired volume of stain and wash buffer suitable for your flow cytometer.
10. Proceed to analyze the samples on a flow cytometer or store at 2-8°C in the dark. For optimal results, do not store the samples longer than 2 hours before acquisition.

Procedural notes

A. Live-dead staining can be performed at the beginning or end of staining procedure according to manufacturer's instructions.
B. Dextramer® staining can be performed on any non-fixed cell suspension, including cell lines, PBMCs, or whole blood. When using red blood cell (RBC) lysis reagents that contain fixative, Dextramer® staining must be performed before RBC lysis.
C. Always keep Dextramer® reagents stored at 2-8°C in the dark – the plastic vial only partially protects the reagents against light.
D. Staining with antibodies may have a negative impact on simultaneous or subsequent staining with Dextramer®. In most cases it is therefore highly recommended to stain with Dextramer® before staining with

antibodies. Simultaneous staining will reduce the Dextramer® staining intensity significantly.

E. Staining can be performed using 96-well microtiter plates. In that case, after antibody incubation make 4 sequential washes using 200 µL stain and wash buffer per well. Centrifuge at 300 x g for 5 min. between each wash and remove supernatant.

Technical support

For additional Tips & Tricks, FAQs and protocols, please visit https://www.immudex.com/resources/ or contact our support team at customer@immudex.com
Telephone: +45 3110 9292 (Denmark), +1 (215) 931 9627 (US).