CAR Dextramer® Staining Protocol

Products

CAR Dextramer®, hCD19 / PE, Cat. No. CT001C PE [size]

Recommended use

CAR Dextramer®, hCD19 is recommended for use in flow cytometry for detection and monitoring of CD19 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 CD19 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 CD19 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®, hCD19 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 cell suspensions, cell lines, TILs, or whole blood, if the cells are non-fixed. For whole-blood samples, stain with Dextramer® reagents before Red Blood Cell (RBC) lysis or use non-fixable RBC lysing solution.

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)