

CAR Dextramer® Staining Protocol

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| Products | CAR Dextramer®, hCD19 / PE, Cat. No. CT001 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) | <p>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.</p> <p>See the FAQ on immudex.com regarding recommended antibody clones. The optimal choice of fluorochromes depends on the flow cytometer and experimental setup.</p> |
| Procedure | <ol style="list-style-type: none"> 1. Prepare your PBMCs or CD19 CAR cells^B by washing twice in 10 mL Stain and Wash buffer. 2. Resuspend 1-3 x 10⁶ PBMCs or 1-2 x 10⁵ 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 | <ol style="list-style-type: none"> 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 μ L 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
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