CD22 CAR Detection Reagent human, Biotin

Order no. 130-126-727

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Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

1. Description

This product is for research use only.

Components	0.06 mL CD22 CAR Detection Reagent, human, Biotin
Capacity	For 30 tests or up to 3×10^7 total cells.
Product format	The reagent is supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The CD22 CAR Detection Reagent, human, Biotin has been developed for the detection of transduced T cells that are engineered to express CD22-specific chimeric antigen receptor (CAR) on the cell surface.

The detection reagent is an antigen-based detection reagent conjugated to biotin. It contains a recombinantly expressed fusion protein consisting of the human CD22 extracellular domains and a specifically mutated human IgG1 Fc region.

The engineered CD22 CAR T cells can be detected via the recognition of the CD22 protein, and identified by flow cytometry via anti-biotin fluorochromes. The mutated human IgG1 Fc region of the CD22 CAR Detection Reagent abolishes their binding to Fcγ receptors. This allows for background-free analysis and eliminates the need for additional blocking steps, such as using a FcR blocking reagent.

1.2 Applications

- Identification and enumeration of CD22 CAR^+ T cells by flow cytometry.

1.3 Recommended reagent dilution

The recommended dilution for CD22 CAR Detection Reagent, human, Biotin is **1:50 for up to 10^6 cells/100 µL**, e.g. 2 µL in a final staining volume of 100 µL for labeling of up to 10^6 cells and subsequent analysis by flow cytometry.

Cells should be stained prior to fixation, if formaldehyde is used as a fixative.

1.4 Reagent requirements

- PEB buffer: autoMACS® Running Buffer (# 130-091-221). Alternatively, prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS Rinsing Solution (# 130-091-222).
- 7-AAD Staining Solution (# 130-111-568 or # 170-080-032, CE-IVD) for dead cell discrimination
- (Optional) Inside Stain Kit (# 130-090-477) for cell fixation
- (Optional) Recommendation of antibodies for staining cocktail. The total volume of the staining cocktail is 100 µL (including Biotin Antibody, PE and 7-AAD Staining Solution):

Product name	Clone
Biotin Antibody, PE, REAfinity	REA746
CD3 Antibody, anti-human, FITC, REAfinity	REA613
CD4 Antibody, anti-human, VioGreen™, REAfinity	REA623
CD8 Antibody, anti-human, APC-Vio® 770, REAfinity	REA734
CD14 Antibody, anti-human, PerCP-Vio 700, REAfinity	REA599
CD45 Antibody, anti-human, VioBlue®, REAfinity	REA747

Table 1: Antibodies which can be used for a staining cocktail.

2. General protocol for immunofluorescent staining

▲ Prepare a staining cocktail containing Biotin Antibody, PE, REAfinity, and 7-AAD Staining Solution for dead cell exclusion, and additional conjugated antibodies. For examples refer to table 1. For details refer to the respective data sheets.

▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ Co-staining with CD22 antibodies, anti-human should be avoided as the CD22 CAR Detection Reagent can also be bound by CD22 antibodies, anti-human.

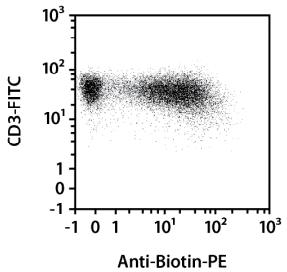
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1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the CD22 CAR Detection Reagent.
   ▲ **Note:** The expression level of CARs might depend on the CAR manufacturing process. For optimal results adjust staining.
5. Mix well and incubate for 10 minutes in the dark at room temperature (19–25 °C).
6. Wash cells by adding 1 mL of buffer per 10^6 cells.
7. Mix well and centrifuge at 300×g for 5 minutes at room temperature (19–25 °C). Aspirate supernatant completely.
8. Add 100 µL of staining cocktail containing Biotin Antibody, PE, REAfinity and 7-AAD Staining Solution, and respective staining antibodies. Mix cells by pipetting up and down.
9. Incubate for 10 minutes in the dark at room temperature (19–25 °C).
10. Wash cells by adding 1 mL of buffer per 10^6 cells.
11. Mix well and centrifuge at 300×g for 5 minutes at room temperature (19–25 °C). Aspirate supernatant completely.
12. (Optional for fixation) Add 250 µL of buffer and 250 µL of Inside Fix to the cells and incubate for 20 minutes in the dark at room temperature (19–25 °C).
13. (Optional for fixation) Add up to 2 mL of buffer.
14. (Optional for fixation) Centrifuge cells at 300×g for 5 minutes at room temperature (19–25 °C). Aspirate supernatant completely.
15. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.
    ▲ **Note:** Acquire the samples within 1 hour after staining.

3. Example of immunofluorescent staining with CD22 CAR Detection Reagent, human, Biotin

CD22 CAR$^+$ T cells were stained with CD22 CAR Detection Reagent, Anti-Biotin-PE, CD3-FITC, and 7-AAD Staining Solution and analyzed by flow cytometry using the MACSQuant® Analyzer 10. Cell debris and dead cells were excluded from the analysis based on scatter signals and 7-AAD fluorescence. Shown are viable CD3$^+$ T cells.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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