Miltenyi Biotec

HER2 CAR Detection Reagent, human, Biotin

Order no. 130-140-083

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1. Description

This product is for research use only.

Components	0.06 mL HER2 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 to +8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

The HER2 CAR Detection Reagent, human, Biotin has been developed for the detection of transduced cells that are engineered to express HER2-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 HER2 extracellular domains and a specifically mutated human IgG1 Fc region.
The engineered HER2 CAR cells can be detected via the recognition of the HER2 protein, and identified by flow cytometry via anti-biotin fluorochromes. The mutated human IgG1 Fc region of the HER2 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 HER2 CAR^+ T cells by flow cytometry.

1.3 Recommended reagent dilution

The recommended dilution for HER2 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

- 7-AAD Staining Solution (# 130-111-568)
- 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).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- (Optional) Double-distilled water (ddH_2O)
- (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, APC-Vio® 770, 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. Protocol

2.1 (Optional) Lysis of whole blood

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O). For example, dilute 2 mL of 10× Red Blood Cell Lysis Solution with 18 mL of ddH_2O.

   ▲ **Note:** Do not dilute with deionized water. Store prepared 1× Red Blood Cell Lysis Solution at room temperature. Discard unused solution at the end of the day.

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2. Add an appropriate amount of whole blood to a suitable tube (e.g. 50 mL or 15 mL capacity).
3. Add 1× Red Blood Cell Lysis Solution in 20-fold excess to the whole blood. For example, add 20 mL of 1× Red Blood Cell Lysis Solution to 1 mL of whole blood.
4. Immediately vortex thoroughly for 3 seconds and incubate for 10–20 minutes in the dark at room temperature (+19 to +25 °C).
5. Centrifuge at 300×g for 10 minutes. Remove supernatant.
6. (Optional) Add at least the 20× volume of PEB buffer to the initial cell sample volume, e.g, add 20 mL buffer when using 1 mL blood. Centrifuge at 300×g for 10 minutes. Remove supernatant.
   ▲ **Note:** An additional washing step reduces staining background.
7. Resuspend cell pellet in a suitable amount of PEB buffer and proceed to immunofluorescence staining (2.2, step 4).

2.2 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.

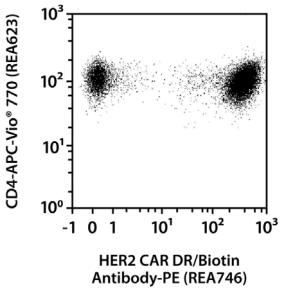
▲ Volumes given below are for **up to 10^6** nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^6 nucleated cells, use twice the volume of all indicated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

▲ Co-staining with HER2 antibodies, anti-human should be avoided as the HER2 CAR Detection Reagent can also be bound by HER2 antibodies, anti-human.

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 PEB buffer.
4. Add 2 µL of the HER2 CAR Detection Reagent.
5. Mix well and incubate for 10 minutes in the dark at room temperature (+19 to +25 °C).
6. Wash cells by adding 1 mL of PEB buffer per 10^6 cells.
7. Mix well and centrifuge at 300×g for 5 minutes at room temperature (+19 to +25 °C). Aspirate supernatant completely.
8. Repeat steps 6 and 7.
9. 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.
10. Incubate for 10 minutes in the dark at room temperature (+19 to +25 °C).
11. Wash cells by adding 1 mL of PEB buffer per 10^6 cells.
12. Mix well and centrifuge at 300×g for 5 minutes at room temperature (+19 to +25 °C). Aspirate supernatant completely.
13. (Optional for fixation) Add 250 µL of PEB buffer and 250 µL of Inside Fix to the cells and incubate for 20 minutes in the dark at room temperature (+19 to +25 °C).
14. (Optional for fixation) Add up to 2 mL of PEB buffer.
15. (Optional for fixation) Centrifuge cells at 300×g for 5 minutes at room temperature (+19 to +25 °C). Aspirate supernatant completely.
16. 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 HER2 CAR Detection Reagent, human, Biotin

HER2 CAR$^+$ SupT1 cells were stained with HER2 CAR Detection Reagent, Anti-Biotin-PE, CD4-APC-Vio 770, 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 CD4$^+$ and CD8$^+$ SupT1 cells.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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