Miltenyi Biotec

BCMA CAR Detection Reagent
human

Contents

1. **Description**
   - 1.1 **Background information**
   - 1.2 **Applications**
   - 1.3 **Recommended reagent dilution**
   - 1.4 **Reagent requirements**
2. **General protocol for immunofluorescent staining**
3. **Example of immunofluorescent staining with BCMA CAR Detection Reagent, human**

1. Description

This product is for research use only.

Components 0.06 mL BCMA CAR Detection Reagent, human

Product	Order no.
BCMA CAR Detection Reagent, human, FITC	130-133-890
BCMA CAR Detection Reagent, human, PE	130-133-888
BCMA CAR Detection Reagent, human, APC	130-136-457
BCMA CAR Detection Reagent, human, Vio® Bright B515	130-136-458
BCMA CAR Detection Reagent, human, Biotin	130-126-090

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 B-cell maturation antigen (BCMA) CAR Detection Reagent, human has been developed for the detection of transduced cells that are engineered to express BCMA-specific chimeric antigen receptor (CAR) on the cell surface. The detection reagent is an antigen-based detection reagent conjugated to various fluorophores. It contains a recombinantly expressed fusion protein consisting of the human BCMA extracellular domains and a specifically mutated human IgG1 Fc region.
The mutated human IgG1 Fc region of the BCMA 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 BCMA CAR$^+$ T cells by flow cytometry.

1.3 Recommended reagent dilution

The recommended dilution for BCMA 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)
- 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) Inside Fix, a component of the Inside Stain Kit (# 130-090-477).
- (Optional) If staining is done with BCMA CAR Detection Reagent, human, Biotin, additionally use Biotin Antibody, REAfinity™ (clone: REA746). The total volume of the staining cocktail is 100 µL (including Biotin Antibody, REAfinity, 7-AAD Staining Solution, and additional flourochrome-conjugated antibodies). Refer to table 1 for a recommendation of antibodies for the staining cocktail.

Productname	Clone
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: Recommended antibodies to include in the staining panel.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-005-815.04

2. General protocol for immunofluorescent staining

▲ 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 BCMA antibodies, anti-human should be avoided as the BCMA CAR Detection Reagent can also be bound by BCMA antibodies, anti-human.

Immunofluorescent staining with Biotin conjugates

▲ Prepare a staining cocktail containing Biotin Antibody, PE, REAfinity, 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.

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 BCMA CAR Detection Reagent, human, Biotin.
5. Mix well and incubate for 10 minutes in the dark (+20 to +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 (+20 to +25 °C). Aspirate supernatant completely.
8. Wash cells again by adding 1 mL of buffer per 10^6 cells.
9. Mix well and centrifuge at 300×g for 5 minutes (+20 to +25 °C). Aspirate supernatant completely.
10. Add 100 µL of staining cocktail containing Biotin Antibody, PE, REAfinity, 7-AAD Staining Solution, and respective staining antibodies. Mix cells by pipetting up and down.
11. Incubate for 10 minutes in the dark (+20 to +25 °C).
12. Wash cells by adding 1 mL of buffer per 10^6 cells.
13. Mix well and centrifuge at 300×g for 5 minutes (+20 to +25 °C). Aspirate supernatant completely.
14. (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 (+20 to +25 °C).
15. (Optional for fixation) Add up to 2 mL of buffer.
16. (Optional for fixation) Centrifuge cells at 300×g for 5 minutes (+20 to +25 °C). Aspirate supernatant completely.
17. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

▲ **Note:** Acquire the samples within 1 hour after staining.

Immunofluorescent staining with fluorochrome conjugates

▲ Prepare a staining cocktail containing BCMA CAR Detection Reagent, human, FITC, PE, APC, or other fluorochromes, 7-AAD Staining Solution for dead cell exclusion, and additional fluorochrome-conjugated antibodies. For details refer to the respective data sheets.

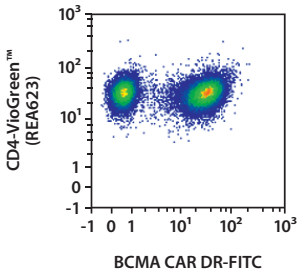
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 50 µL of buffer.
4. Add 50 µL staining cocktail containing 2 µL BCMA CAR Detection Reagent, human, FITC, PE, APC, or other fluorochromes, 7-AAD Staining Solution, and additional fluorochrome-conjugated antibodies.
5. Mix well by pipetting up and down and incubate for 10 minutes in the dark (+20 to +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 (+20 to +25 °C). Aspirate supernatant completely.
8. (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 (+20 to +25 °C).
9. (Optional for fixation) Add up to 2 mL of buffer.
10. (Optional for fixation) Centrifuge cells at 300×g for 5 minutes (+20 to +25 °C). Aspirate supernatant completely.
11. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

▲ **Note:** Acquire the samples within 1 hour after staining.

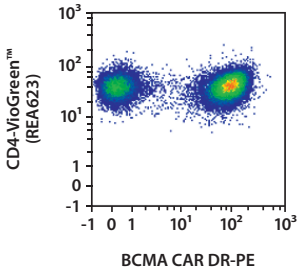
3. Example of immunofluorescent staining with BCMA CAR Detection Reagent, human

BCMA CAR$^+$ SupT1 cells were stained with BCMA CAR Detection Reagent, human, FITC (A), PE (B), APC (C), Vio Bright B515 (D), or Biotin (E), Anti-Biotin-PE, CD4-VioGreen, CD8-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.

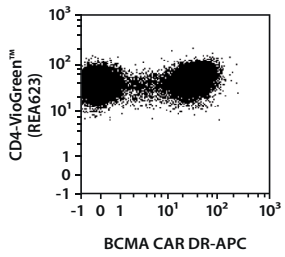
A)



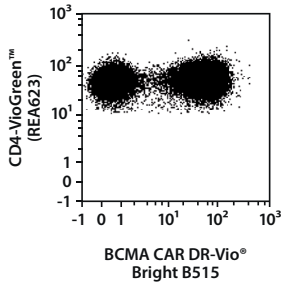
B)



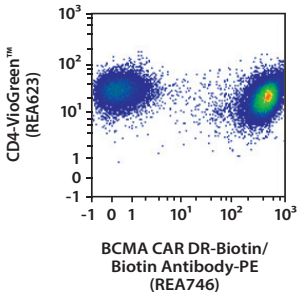
C)



D)



E)



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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, MACS, MACSQuant, the Miltenyi Biotec logo, REAfinity, Vio, VioBlue, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Copyright © 2024 Miltenyi Biotec and/or its affiliates. All rights reserved.