Miltenyi Biotec

StainExpress™ CAR T Transduction Cocktail

human

Order no. 130-127-638

Contents

1. Description

This product is for research use only.

Components	**24 tubes StainExpress CAR T Transduction Cocktail, human:** Dried cocktail of fluorochrome-conjugated recombinant engineered REAfinity™ Antibodies (isotype: recombinant human IgG1) containing: CD45-VioBlue® (clone: REA747), CD4-VioGreen™ (clone: REA623), CD3-FITC (clone: REA613), CD14-APC (clone: REA599), CD8-APC-Vio® 770 (clone: REA734), 7-AAD Staining Solution. **2 StainExpress CAR T Transduction Compensation Sets, human**: Each set contains five dried single antibody tubes of fluorochrome-conjugated recombinant engineered REAfinity Antibodies (isotype: recombinant human IgG1) for compensation controls: CD45-VioBlue (clone: REA747), CD4-VioGreen (clone: REA623), CD3-FITC (clone: REA613), CD14-APC (clone: REA599), CD8-APC-Vio 770 (clone: REA734).
Capacity	24 tests, one test for up to 10^6 total cells.
Product format	Antibodies are supplied in a dry format containing stabilizer.
Storage	Store at dry conditions in a closed pouch. Store protected from light at 19–25 °C. The expiration date is indicated on the pouch label.

1.1 Background information

The StainExpress CAR T Transduction Cocktail provides a pre-formulated antibody backbone panel to simplify flow cytometric evaluation of transduction efficiency in cell manufacturing of chimeric antigen receptor $(CAR)^+$ T cells. This unified backbone panel includes a free PE channel for detection of the CAR Detection Reagent of choice. The Miltenyi Biotec CAR Detection Reagents have been developed for the detection of transduced T cells that are engineered to express specific CARs on the cell surface.
For flow cytometric analysis use a flow cytometer equipped with a red (638 nm), a blue (488 nm), and a violet (405 nm) laser, for example, the MACSQuant Analyzer 16.

1.2 Applications

- Evaluation of the transduction efficiency in CAR T cell manufacturing.
- Identification of CAR^+ T cells by flow cytometry.

1.3 Reagent and instrument requirements

- Buffer: 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). Keep buffer cold (2–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Flow cytometer, e.g., MACSQuant Analyzer 16 (# 130-109-803) or MACSQuant Analyzer 10 (# 130-096-343).

  ▲ **Note:** The MACSQuant VYB cannot be used.

- CAR Detection Reagent of choice detectable in the PE channel, e.g., CD19 CAR Detection Reagent, human, Biotin (# 130-129-550), CD19 CAR FMC63 Idiotype Antibody, PE, REAfinity (130-127-342), CD22 CAR Detection Reagent, human, Biotin (# 130-126-727), or BCMA CAR Detection Reagent, human, PE (# 130-133-888).
- (Optional) Biotin Antibody, PE, REAfinity (# 130-110-951).
- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693), for compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.
- (Optional) MACS MiniSampler Plus (# 130-105-745).
- (Optional) Chill 5 Rack (# 130-092-951).
- (Optional) CAR T Cell Express Mode Package (# 160-002-376).

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2. Protocols

2.1 Immunofluorescent staining of nucleated cells, e.g., T cells

▲ 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, use multiple tubes accordingly (e.g. for 2×10^6 nucleated cells, use two tubes).

1. Determine cell number.
2. Adjust cell concentration to up to 10^6 nucleated cells per 100 µL using buffer.

   ▲ **Note:** If necessary, centrifuge cell suspension at 300×g for 5 minutes, aspirate supernatant completely, and resuspend up to 10^6 nucleated cells per 100 µL of buffer.
3. Add 100 µL of cell suspension to one tube of StainExpress CAR T Transduction Cocktail.
4. Add suitable amount of CAR Detection Reagent.

   ▲ **Note:** For example, if using CD19 CAR Detection Reagent, human, Biotin the suitable amount is 2 µL.
5. Incubate for 10–20 seconds, then vortex at high speed for 5 seconds and incubate for 10 minutes in the dark at 19–25 °C.

   ▲ **Note:** Working at lower temperatures requires increased incubation times.
6. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
7. (Optional) If using a biotin-conjugated CAR Detection Reagent follow steps 7a–d:

   a. Repeat step 6.

   b. Resuspend cells in 98 µL buffer and add 2 µL of Biotin Antibody, PE, REAfinity.

   c. Vortex shortly at high speed and incubate for 10 minutes in the dark at 19–25 °C.

   d. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

   ▲ **Note:** Store samples at 2–8 °C protected from light until analysis. Acquire samples within 1 hour after staining.
9. Proceed to flow cytometric analysis.

2.2 Flow cytometric data acquisition with the MACSQuant Analyzer 16 using an Express Mode

▲ Please refer to the MACSQuant Instrument user manual and software guide for detailed information on using the MACSQuant Analyzer.

▲ Please refer to the data sheet of the MACS Comp Bead Kit, anti-REA, when using beads for compensation.

1. Prepare and prime the MACSQuant Analyzer. Make sure the calibration and instrument settings of the instrument have been optimized for acquisition of the StainExpress CAR T Transduction Cocktail.
2. For optimal compensation, prepare single stainings of suitable beads or cells with all single antibody tubes from one pouch of the matching StainExpress Compensation Set. If using beads, add one full drop of MACS Comp Beads – anti-REA and one full drop of MACS Comp Beads – blank directly to each compensation tube.
   (Optional) If using a biotin-conjugated CAR Detection Reagent prepare an additional tube for optimal compensation of the used antibody conjugate. For example, if using CD19 CAR Detection Reagent, human, Biotin in combination with Biotin Antibody, PE, REAfinity, prepare an additional tube with 98 µL buffer and add 2 µL of Biotin Antibody, PE, REAfinity.

   ▲ **Note:** For optimal compensation always use the provided StainExpress Compensation Set matching the StainExpress Cocktails used. To ensure they match, check for identical order number-related lot numbers indicated on the pouches next to the expiration date.

   ▲ **Note:** One full drop of beads is approximately 50 µL.

   ▲ **Note:** For compensation on cells add 100 µL cell suspension instead of beads directly to each compensation tube.
3. Incubate for 10–20 seconds, then vortex at high speed for 5 seconds and incubate for 10 minutes in the dark at 19–25 °C.

   ▲ **Note:** Working at lower temperatures requires increased incubation times.
4. Dilute each sample by adding 600 µL of MACSQuant Running Buffer. Mix well.
5. If using the MACS Comp Bead Kit, anti-REA, follow the protocol for compensation set up of the MACSQuant Analyzer.

   ▲ **Note:** For automated compensation choose the "CompensationMultiColor" Express Mode.
6. Choose appropriate voltage settings for forward scatter (FSC) and side scatter (SSC).
7. Define an appropriate threshold, based on FSC versus SSC, for the exclusion of debris from the data acquisition.
8. Select the corresponding Express Mode.
9. Start flow cytometric data acquisition.

3. Examples of immunofluorescent staining with the StainExpress CAR T Transduction Cocktail

CAR T cells, generated within 12 days using the CliniMACS Prodigy® T Cell Transduction process, were stained with the StainExpress CAR T Transduction Cocktail, CD19 CAR Detection Reagent, human, Biotin, and Biotin Antibody, PE, REAfinity. Staining was carried out at 19–25 °C for 10 minutes. Cells were analyzed by flow cytometry using the MACSQuant Analyzer 10. To exclude debris, a gate was set on FSC versus SSC encompassing all cells (A). To exclude residual erythrocytes and to identify leukocytes, CD45 was used to gate on $CD45^+$ leukocytes (B). Dead cells were excluded by gating on 7-AAD^- cells (C). $CD3^+$ cells were identified (D) and discriminated in CAR^+ and CAR^- cells (E). CAR^+ cells were further divided into $CD4^+$ and $CD8^+$ T cells (F), as were CAR^- cells (G). Among $CD3^-$ cells, residual monocytes were defined by CD14 expression (H).

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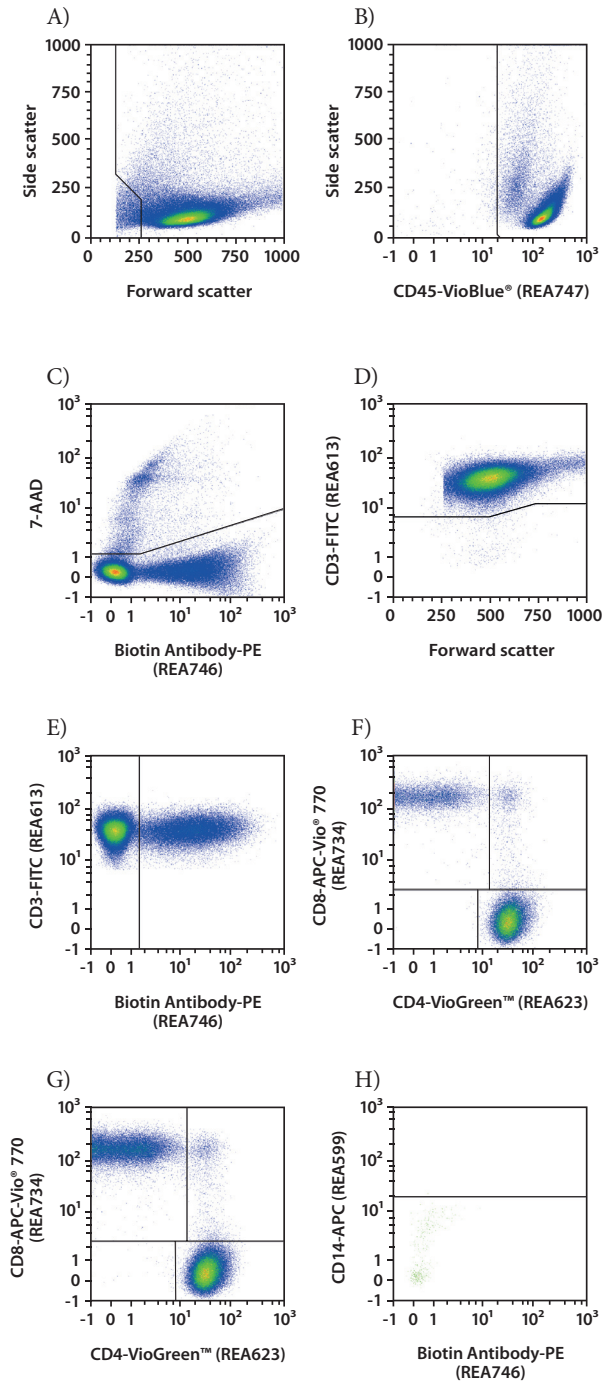

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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