Miltenyi Biotec

StainExpress™ Immune Cell Composition Cocktail

human

Order no. 130-127-637

Contents

1. Description

This product is for research use only.

Components

24 tubes StainExpress Immune Cell Composition 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),
CD56-PE (clone: REA196),
CD16-PE (clone: REA423),
CD19-PE-Vio® 770 (clone: REA675),
CD14-APC (clone: REA599),
CD8-APC-Vio 770 (clone: REA734),
7-AAD Staining Solution.

2 StainExpress Immune Cell Composition Compensation Sets, human:

Each set contains seven 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),
CD16-PE (clone: REA423),
CD19-PE-Vio 770 (clone: REA675),
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 Immune Cell Composition Cocktail simplifies flow cytometric evaluation of cell fractions for immunofluorescent staining of whole blood, leukapheresis products, peripheral mononuclear cells (PBMCs), or other single cell suspensions from human tissue.
The cocktail has been designed for the reliable identification of human monocytes, neutrophils, eosinophils, and T, B, and NK lymphocyte populations as well as $CD4^+$, $CD8^+$, and NKT cell subsets in human blood.
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 leukocyte subsets in whole blood, leukapheresis products, PBMCs, or other single cell suspensions from human tissue.
- Determination of cell counts and population subsets during CAR T cell manufacturing procedures using the MACSQuant Analyzer.

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.

- (Optional) MACS Comp Bead Kit, anti-REA (# 130-104-693), for compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.
- (Optional) 10× Red Blood Cell Lysis Solution (# 130-094-183)
- (Optional) MACS MiniSampler Plus (# 130-105-745)

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- (Optional) Chill 5 Rack (# 130-092-951)
- (Optional) CAR T Cell Express Mode Package (# 160-002-376)

2. Protocols

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

▲ 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 Immune Cell Composition Cocktail.
4. 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.
5. (Optional) Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. 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.
7. Proceed to flow cytometric analysis.

2.2 Immunofluorescent staining and lysis of whole blood or leukapheresis products (lyse/no wash)

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 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.
2. Add 100 µL of whole blood or leukapheresis product to one StainExpress Immune Cell Composition Cocktail 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. Add 1900 µL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 3 seconds. Incubate for 15 minutes in the dark at 19–25 °C.

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

2.3 Immunofluorescent staining and lysis of whole blood or leukapheresis products (lyse/wash)

1. Dilute 10× Red Blood Cell Lysis Solution 1:10 with double-distilled water (ddH_2O), for example, dilute 1 mL of 10× Red Blood Cell Lysis Solution with 9 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.
2. Add 100 µL of whole blood or leukapheresis product to one StainExpress Immune Cell Composition Cocktail 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. Add 1900 µL of 1× Red Blood Cell Lysis Solution and immediately vortex thoroughly for 3 seconds. Incubate for 15 minutes in the dark at 19–25 °C.
5. Centrifuge at 300×g for 5 minutes. Aspirate supernatant completely.
6. 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.
7. Proceed to flow cytometric analysis.

2.4 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 Immune Cell Composition 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.

   ▲ **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.

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5. If using the MACS Comp Bead Kit, anti-REA, follow the protocol for compensation set up of the MACSQuant Analyzer.

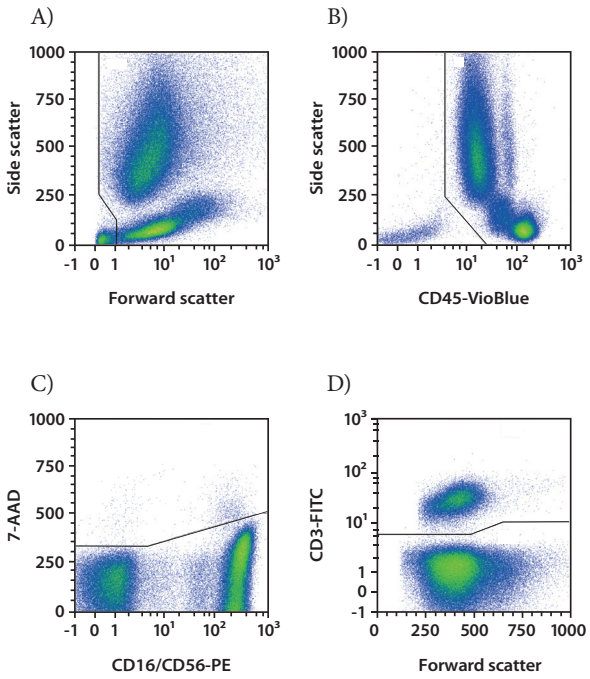
   ▲ **Note:** For automated compensation choose the "CompensationMultiColor" Express Mode.

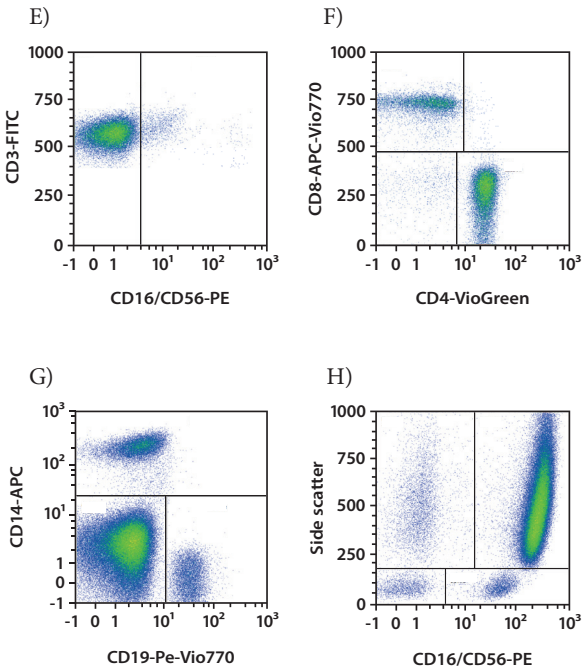
6. Choose appropriate voltage settings for forward scatter (FSC) and side scatter (SSC).
7. Define an appropriate threshold, based on FSC versus SSC, for the exclusion of debris from the data acquisition.
8. Select the corresponding Express Mode.
9. Start flow cytometric data acquisition.

3. Examples of immunofluorescent staining with the StainExpress Immune Cell Composition Cocktail

Whole blood from a healthy donor was stained with the StainExpress Immune Cell Composition Cocktail. Staining was carried out for 10 minutes at room temperature (19–25 °C). Subsequently, red blood cells were lysed by incubation with 1× Red Blood Cell Lysis Solution at room temperature for 15 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), and dead cells were excluded by 7-AAD (C). $CD3^+$ cells were identified (D) and discriminated into T cells and NKT cells based on expression of CD56 (E). T cells were further divided into $CD4^+$ and $CD8^+$ T cells (F). Among $CD3^-$ cells, monocytes were defined by CD14 expression and B cells by CD19 expression (G). The remaining $CD14^-/CD19^-$ cells were further devided into $SSC^{high}/CD16^-$ eosinophils, $SSC^{high}/CD16^+$ neutrophils, and $SSC^{low}/CD56^+/CD16^+$ cells (H).





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