Miltenyi Biotec

Tandem Signal Enhancer
human

0.3 mL Order no. 130-099-887
1 mL Order no. 130-099-888

Contents

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	**0.3 mL of Tandem Signal Enhancer** **or** **1 mL of Tandem Signal Enhancer**
Capacity	300 µL: 30 tests of PBMCs and whole blood or up to 3×10^8 total cells 1 mL: 100 tests of PBMCs and whole blood or up to 10^9 total cells
Product format	All products are 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

When used for labeling human peripheral blood mononuclear cells (PBMCs) or whole blood tandem-dye–conjugated antibodies often show non-specific binding, especially to monocytes. The Tandem Signal Enhancer strongly reduces non-specific binding of tandem conjugates. Accordingly, negative cells show decreased background staining, which results in higher stain indices for target cell populations. The additional use of FcR Blocking Reagent is not required.

1.2 Applications

- Blocking of tandem-dye–conjugated antibodies binding to monocytes and non-target cells. Suitable for tandem dyes, such as PerCP-Vio® 700, PerCP-Cy® 5.5, PE-Cy5.5, PE-Vio 770, PE-Cy7, APC-Vio 770, APC-Cy7, APC-H7, ECD, PE-Vio 615, BD Horizon™ PE-CF594, PE/Dazzle™ 594, or PE-eFluor® 610.

1.3 Recommended antibody dilution

The recommended Tandem Signal Enhancer dilution is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

1.4 Reagent 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.

- Red Blood Cell Lysis Solution (10×) (# 130-094-183).
- (Optional) For labeling of human whole blood: MACSQuant® Storage Solution (# 130-092-748).
- (Optional) For labeling of human whole blood: fluorochrome-conjugated CD45 Antibody, anti-human. For antibodies for additional staining refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMCs should be isolated by density gradient centrifugation, for example, using Ficoll-Paque™.

▲ **Note:** To remove platelets after density gradient separation, resuspend cell pellet in buffer and centrifuge at 200×g for 10–15 minutes at 20 °C. Carefully aspirate supernatant. Repeat washing step.

When working with tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

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▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 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 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before labeling. Pass cells through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

2.2 Use of Tandem Signal Enhancer and antibodies for the labeling of human PBMCs

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 90 µL of buffer.

   ▲ **Note:** If staining with several antibodies (see step 5), adjust the volume of buffer accordingly. Recommended dilution is 1:11.
4. Add 10 µL of Tandem Signal Enhancer.
5. Add staining antibodies as recommended by the manufacturer. Using MACS Antibodies add the antibody according to the respective data sheet.

   ▲ **Note:** If staining with several antibodies, adjust the volume of buffer accordingly. Recommended dilution is 1:11.
6. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
7. Wash cells by adding 1 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

2.3 Use of Tandem Signal Enhancer and antibodies for the labeling of human whole blood

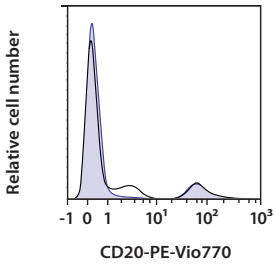
1. Prepare a 4× lysis solution by diluting Red Blood Lysis Solution (10×) 1:2.5 with double-distilled water (ddH_2O).
2. Pipette all conjugates into a reaction vessel in a titer as recommended by the manufacturer. For a better enumeration always use CD45 antibodies for triggering.
3. Add 10 µL of Tandem Signal Enhancer.
4. Adjust volume to 60 µL with buffer.

   ▲ **Note:** If staining with several antibodies, adjust the volume of buffer accordingly. Recommended dilution is 1:11.
5. Add 50 µL of whole blood.

   ▲ **Note:** Best suitable is whole blood supplemented with EDTA or Heparin.
6. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
7. Add 250 µL of 4× lysis solution and 750 µL of MACSQuant Storage Solution.
8. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).
9. Use lysed blood directly (lyse – no wash) in flow cytometry. Alternatively, centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely (lyse – wash).
10. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Example of immunofluorescent staining on whole blood using the Tandem Signal Enhancer

Fresh lysed human whole blood was stained with CD45 antibodies for targeting on all $CD45^+$ cells and analyzed using the MACSQuant Analyzer. Then, a gate was set on all lymphocytes and monocytes. The histogram shows staining with CD20-PE-Vio 770 without supplement (black line) and with the use of Tandem Signal Enhancer (purple line).



Conjugate	Stain index without Tandem Signal Enhancer	Stain index with Tandem Signal Enhancer
CD20-PE-Vio 770	37.4	87.2

For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

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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