Miltenyi Biotec

Advanced flow cytometry

FASER Kit – FITC

Order No. 130-091-763

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1. Description

This product is for research use only.

Components	2×2 mL FcR Blocking Reagent, 1 mL FITC-Activator (Reagent 1), 1 mL FITC-Enhancer (Reagent 2).
Size	For 10^9 total cells, up to 100 stainings.
Staining concentration	FcR Blocking Reagent is used at a dilution of 1:5. The FITC-Activator (Reagent 1) and the FITC-Enhancer (Reagent 2) are used at a dilution of 1:11.
Product format	Reagents are supplied as a solution containing 0.1% gelatine and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The FASER (Fluorescence Amplification by Sequential Employ-ment of Reagents) Kit – FITC (fluorescein-isothiocyanate) is developed to increase the fluorescence intensity of cells labeled with a FITC-conjugated antibody. The intensity of the FITC fluorescence is amplified by sequential addition of the FITC-Activator (Reagent 1) and the FITC-Enhancer (Reagent 2). For further enhance-ment of the fluorescence intensity, sequential addition of the FITC-Activator (Reagent 1) and the FITC-Enhancer (Reagent 2) can be repeated several times.

The FASER Kit – FITC does not affect direct staining with other fluorochrome-conjugated antibodies, but should not be combined with indirect immunofluorescent staining using biotin-conjugated antibodies. The Kit is suitable for FITC-labeled, fresh or formaldehyde-fixed cells of any type or species in suspension. Analysis is performed by flow cytometry.

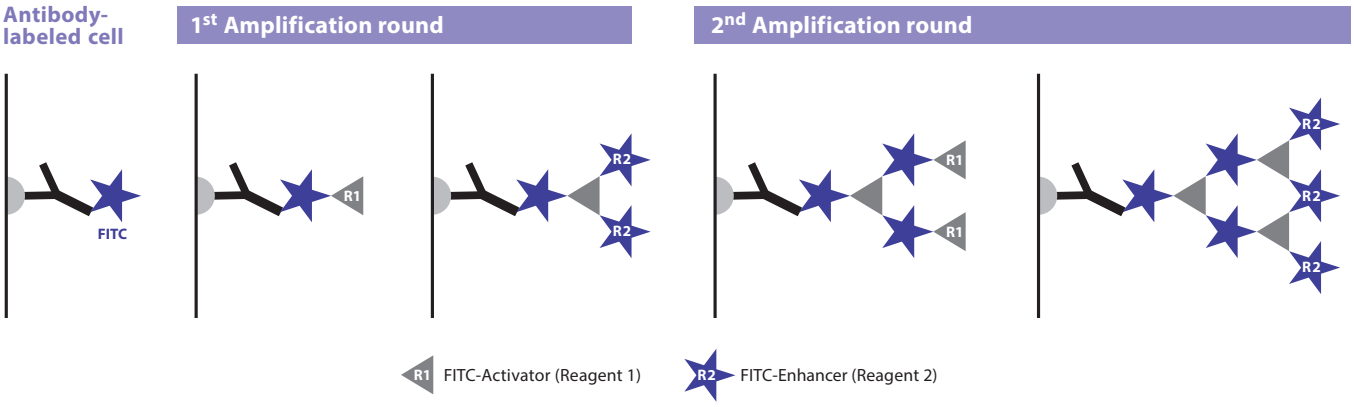
Examples of product application

- Amplification of FITC fluorescence which is weak, for example
  - due to FITC-conjugated antibody staining of antigens which are expressed at low levels,
  - due to immunomagnetic and FITC-conjugated antibody labeling of the same antigen epitope,
  - due to FITC-conjugated antibody staining with a low affinity antibody.
- Amplification of the fluorescence signal of a FITC-labeled cell fraction for a clearer flow cytometric discrimination from the non-labeled cell fraction.
- Amplification of magnetic labeling with Anti-FITC MicroBeads (# 130-048-701) by increasing the number of Anti-FITC MicroBead binding sites per cell.

1.2 Reagent requirements

- Buffer: PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% BSA (bovine serum albumin) and 2 mM EDTA. Keep buffer cold (4–8 °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 serum albumin, serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.



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140-001-189.03

2. General protocol for immunofluorescent staining

▲ If formaldehyde fixation of cells is required, cells should be stained and treated with the FASER Kit – FITC prior to fixation.

▲ If the FASER Kit – FITC is used for amplification of magnetic labeling with Anti-FITC MicroBeads, amplify FITC staining as described below. For subsequent magnetic labeling, refer to the Anti-FITC MicroBead data sheet.

▲ Volumes for fluorescent labeling given below are for up to 10^7 total 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 total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Label cells with desired FITC-conjugated antibody and afterwards wash cells according to the manufacturer's recommendations.
2. Resuspend up to 10^7 cells in 80 µL of buffer.
3. Add 20 µL of **FcR Blocking Reagent**.
4. Add 10 µL of **FITC-Activator (Reagent 1)**.
5. Mix well and incubate for 10 minutes in the dark at 4–8 °C.

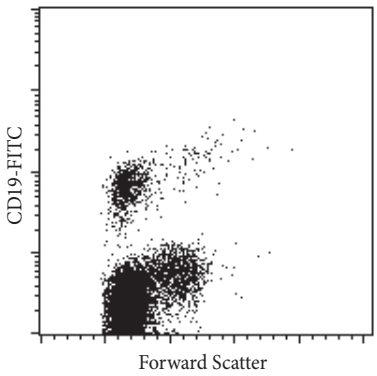
   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
7. Resuspend cell pellet in 80 µL of buffer.
8. Add 20 µL of **FcR Blocking Reagent**.
9. Add 10 µL of **FITC-Enhancer (Reagent 2)**.
10. Mix well and incubate for 10 minutes in the dark at 4–8 °C.

    ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
11. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
12. (Optional) Repeat steps 2–11, if further amplification of the FITC fluorescence is required.
13. Finally resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry.

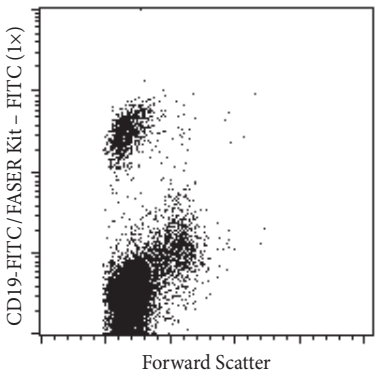
3. Example of immunofluorescence amplification using the FASER Kit – FITC

Human peripheral blood mononuclear cells (PBMC) were stained with CD19-FITC, human (# 130-091-328), and the FITC fluorescence was increased by two rounds of amplification using the FASER Kit – FITC. Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide (PI) fluorescence.

Before use of the FASER Kit – FITC

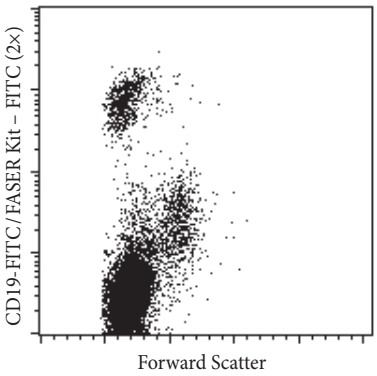


After one round of amplification using the FASER Kit – FITC



The mean fluorescence intensity of the $CD19^+$ cell population is amplified by a factor of 6 compared to the non-amplified staining.

After a second round of amplification using the FASER Kit – FITC



The mean fluorescence intensity of the $CD19^+$ cell population is amplified by a factor of 9 compared to the non-amplified staining.

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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