Miltenyi Biotec

# MACSelect™ Control FITC Antibody
## human

Order no. 130-090-326

## Contents


1. **Description**
   - 1.1 **Background information**
   - 1.2 **Applications**
   - 1.3 **Reagent and instrument requirements**
2. **General protocol for immunofluorescent staining**
3. **Example of immunofluorescent staining with MACSelect™ Control FITC**


## 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** | 1 mL FITC-conjugated antibody:<br>Polyclonal antibody which fluorescently stains MACSelect MicroBeads.. |
| **Capacity** | 100 tests. |
| **Product format** | Antibodies 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. |

The MACSelect Control FITC Antibody has been tested to cross-react with B cells.

### 1.1 Background information

The MACSelect Control FITC Antibody is used to determine the enrichment efficiency of pMACS vector-transfected cells. Transfected cells are magnetically labeled with MACSelect MicroBeads. The fluorescent staining with MACSelect Control FITC Antibody is performed after magnetic labeling with MACSelect 4, MACSelect $K^k$, or MACSelect LNGFR MicroBeads. To determine the enrichment efficiency, the MACSelect Control FITC-stained cells are analyzed by flow cytometry or fluorescence microscopy. By comparing the amount of fluorescently labeled cells before and after magnetic separation, the enrichment rate, which is achieved with the MACSelect System, can be determined.

### 1.2 Applications

- Identification and enumeration of MACSelect Control FITC-stained cells by flow cytometry or fluorescence microscopy.
- Fluorescent staining of magnetically labeled, transfected cells to determine the enrichment rate achieved with MACSelect.

### 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.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

## 2. General protocol for immunofluorescent staining

▲ The MACSelect Enrichment rate of transfected cells can vary with the individual experimental design that is used (e.g., cell line and transfection method).

▲ Most adherent cells are rather large and should be centrifuged at a maximum speed of 50–100×g. At a higher speed they may burst. The larger the cells are, the lower the centrifugation speed should be. When centrifuging suspension cells, the centrifugation speed should not exceed 200×g.

▲ Perform the microscopic evaluation directly after fluorescent staining. A delay of the analysis may result in a decrease in fluorescent intensity.

▲ The enrichment rate ($f_E$) represents the factor by which the ratio of marker positive cells versus marker negative cells is increased after the enrichment. Therefore $f_E$ represents the average number of negative cells that pass through the column per negative cell retained unspecifically (assuming that all positive cells are retained). This value is low if cells are unspecifically labeled and retained.

$$\frac{\%\text{ pos. cells in orig. sample}}{\%\text{ neg. cells in orig. sample}} \times f_E = \frac{\%\text{ pos. cells in pos. fraction}}{\%\text{ neg. cells in pos. fraction}}$$

$$\rightarrow f_E = \frac{\%\text{ neg. cells in orig. sample}}{\%\text{ pos. cells in orig. sample}} \times \frac{\%\text{ pos. cells in pos. fraction}}{\%\text{ neg. cells in pos. fraction}}$$

1. Transfect cells with pMACS vector which contains your gene of interest using your standard transfection protocol. Use 1–20 µg of the pMACS plasmid depending on the transfection method, the cell number, the cell type, and the amount of cells used.
2. After transfection, culture the cells for an appropriate time as determined previously. For details see MACSelect user manual.


**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**


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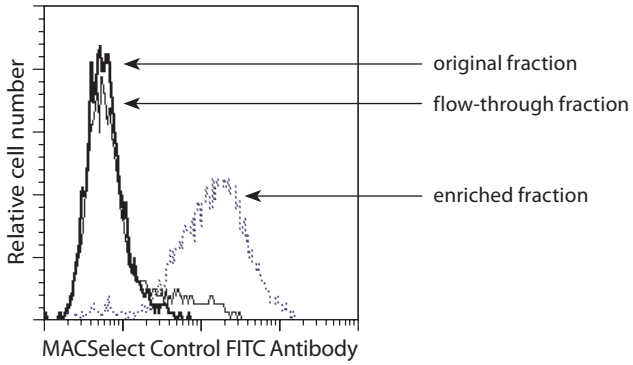
3. Remove an aliquot of the magnetically labeled, transfected cells for subsequent fluorescent staining (original fraction). Then proceed to magnetic separation as described.
4. After magnetic separation remove an aliquot of the eluted cells for fluorescent staining (positive fraction).
5. Centrifuge both fractions, i.e. original and positive fraction, for 5 minutes at 50–200×g at room temperature.
6. Aspirate supernatant completely and resuspend the cells in 100 µL of buffer.
7. Add 10 µL of MACSelect Control FITC Antibody.

   ▲ **Note:** MACSelect Control FITC Antibody can only be used in combination with MACSelect MicroBeads. It is not suitable for fluorescent staining of other MACS MicroBeads.
8. Mix well and incubate for 5–10 minutes in the dark in the refrigerator (2–8 °C).

   ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
9. Wash cells by adding 1–2 mL of buffer and centrifuge at 50–200×g for 5 minutes at room temperature.
10. For flow cytometric analysis: Resuspend cells in an appropriate amount of buffer and proceed to analysis. For fluorescence microscopic analysis: Resuspend cell pellet without adding further buffer and transfer the resuspended cells to a glass slide. Cover the cells with a cover slip and proceed immediately to microscopic analysis.

## 3. Example of immunofluorescent staining with MACSelect™ Control FITC

CHO cells were transfected with pMACS $K^k$.II. After 20 h of incubation, the cells were magnetically labeled with MACSelect $K^k$ MicroBeads and separated using an MS Column. Aliquots of the different cell fractions were fluorescently stained with MACSelect Control FITC Antibody and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.




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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