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1. Description

Components	1 mL monoclonal Anti-Feeder antibodies, mouse conjugated to various dyes.
	PE 130-096-094
	APC 130-096-099
	Biotin 130-096-095
Clone	mEF-SK4 (isotype: rat IgG1).
Capacity	100 tests or up to 10 ⁹ total cells.
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.

1.1 Background information

The most prevalent cultivation method for maintenance of mouse and human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells is co-cultivation on primary mouse embryonic fibroblast (mEF) feeder cell layers. Feeder cell layers of NIH3T3 fibroblasts or stromal derived OP9 cells are also frequently used in co-culture systems: NIH3T3 fibroblasts support primary keratinocyte cultures, OP9 cells are used for hematopoietic induction of ES cells.

1.2 Applications

- Discrimination (gating out) of mouse feeder cells by flow cytometry allows analysis of pure stem cells.
- Identification and enumeration of cultivated mouse feeder cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse feeder cells can be depleted by using Feeder Removal MicroBeads, mouse (# 130-095-531).

1.3 Recommended antibody dilution

The recommended antibody dilution for all Anti-Feeder conjugates is **1:11 for up to 10⁷ cells/100 µL** of buffer for labeling of cells and analysis by flow cytometry. For Feeder Removal MicroBead-labeled cells use the same dilution.

The antibody is suited for staining of formaldehyd-fixed cells. For optimal results, cells must be stained after to fixation with formaldehyde.

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 a mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin antibodies conjugated to, e.g., PE (# 130-090-756) or VioBlue® (# 130-094-669) as secondary antibody reagent in combination with Anti-Feeder-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10⁷** nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

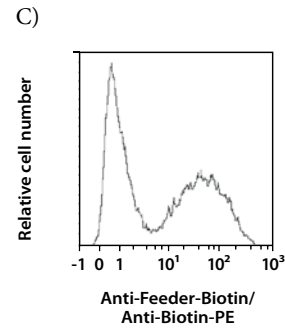
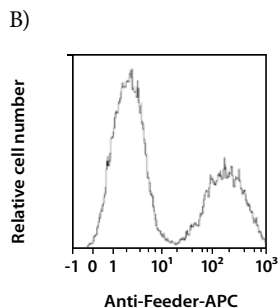
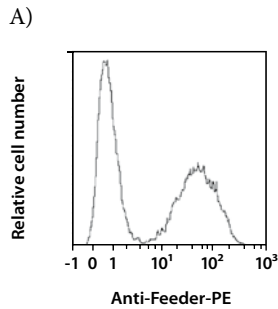
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
4. Add 10 µL of the Anti-Feeder antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.

6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If Anti-Feeder-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, and continue as described in steps 5 and 6. Repeat step 6.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-Feeder antibodies

Co-cultures of mouse embryonic stem (mES) cells and mouse embryonic fibroblast (mEF) cells were stained with Anti-Feeder antibodies conjugated to PE (A) or APC (B) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with Anti-Feeder-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756). mEF cells can be clearly discriminated from unstained mES cells based on the Anti-Feeder antibody staining. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. Reference

1. Knoebel, S. *et al.* (2010) ISSCR 8th annual Meeting. Abstract 2010-P1256-ISSCR.

All protocols and data sheets are available at www.miltenyibiotec.com.

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.

Warranty

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