# XpresSep™ Rat anti-Mouse $IgG_1$ Ferrofluid (RAM-FF) Kit for Custom Indirect Separations

Catalog #: RAMFF-109 Capacity: 1 x $10^9$ cells

## 1. Product Description

**Materials**

| | |
|---|---|
| **Supplied** | • 0.5 mL XpresSep™ RAM-Ferrofluid |
| | • 10mL 10X Buffer |
| **Required** | • $ddH_2O$ |
| | • $IgG_1$ Monoclonal Antibodies |
| | • XpresSep™ Multipole Magnetic Separator |
| **Storage and Stability** | Materials may be shipped at room temperature, but should be stored protected from light at 4–8°C upon receipt. Do not freeze. The expiration date is indicated on the vial label. |

## 2. Product applications

- RAM-FF is particularly well suited for an indirect-label, negative selection of cells from apheresis, PBMC, or cell culture suspensions as unbound mAb need not be removed following incubation with labeling mAb(s). Labeling mAbs must be mouse $IgG_1$ and up to a total of 6 µg of a mAb cocktail can be used per $10^8$ cells.
- Starting with fresh PBMC, the enriched fraction typically contains between 96.5% - 99.9% selected cells.

## 3. Protocol

### I. Reagent/Cell Preparation

**a.** **Buffer:** Dilute 10x with $ddH_2O$ and store at 2-8˚C when not in use.

**b.** **mAb or mAb cocktail:** Dilute the antibody(ies) of interest in 500 µL buffer. The amount of each antibody can range from 1.2 to 3 µg/mL while the total amount of antibodies in the cocktail should not exceed 12 µg/mL in buffer.

**c.** **RAM-FF:** For each mL of cells to be processed, mix 48 µL of RAM-FF with 952 µL of buffer. Keep on ice prior to use.

**d.** **Cell suspension:** Prepare at 2 x $10^8$ cells/mL in buffer.

### II. Optimum Conditions for Indirect Negative Selection Ferrofluid Separations

**a.** mAb incubation: mix equal volumes of mAb(s) (1.2 - 12 µg /mL) and cells (2 x $10^8$/mL). Mix gently by inversion. Incubate at room temperature for 10-15 min.

**b.** Following mAb incubation, mix equal volumes of cell suspension and RAM-FF solution.

**c.** Incubate 10 -15 min.

**d.** Dilute cells to 2 x $10^7$ cells/mL for magnetic separation.

**e.** Place the cell mixture in the appropriate XpresSep™ Multipole Magnetic Separator and incubate for 10 min.

**i.** 1 mL – 4 mL → 12x75 tube: XpresSep™5

**ii.** >4 mL – 14 mL → 15 mL tube: XpresSep™15

**iii.** >14 mL – 45 mL → 50 mL tube: XpresSep™50

**f.** Carefully harvest the supernatant using a Pasteur pipette.

**g.** In the event yield of negatively selected cells is below 75% when large numbers of cells are being removed, it is possible to increase yields by removing separation tube from the magnet, adding cell buffer, and gently resuspending cells prior to performing a 2nd separation. In that way cells that might have been entrained during separation can often be recovered.

### III. When to Remove Unbound mAb

**a.** With very high affinity mAbs as many as 10 mAbs can be included in a cocktail in which case not more than 6 µg of mAb is added to 1 x $10^8$ cells. In the event that more than this amount of mAb is required, unbound mAb should be removed by centrifugation before adding RAM-FF.

**b.** If necessary, dilute the cell-mAb mixture (3-4X) with buffer and centrifuge at 300 xg for 10 min. Remove the supernatant, being careful to avoid the cell pellet, and resuspend the cells to their original volume with buffer. Proceed as above with RAM-FF addition, incubation, and separation.

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## Safety

The XpresSep™ Ferrofluid contains 0.05% Proclin™ 300. This concentration presents no health hazards, toxicology problems, or disposal issues. Please consult the Safety Data Sheet for additional information.

Products are for **RESEARCH USE ONLY** and are not intended for human or animal therapeutic or diagnostic uses.

## Warranty

The products are warrantied only against defects in workmanship and quality at the time of delivery. BioMagnetic Solutions LLC makes no warranty beyond the technical specifications of the product. BioMagnetic Solutions LLC liability is limited to either replacement of the products or refund of the purchase price.



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