

# ImmunoCult™-ACF Human B Cell Expansion Supplement

**Serum-free and animal component-free culture supplement for expansion of human B cells**

Catalog #10974

2 mL



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## Product Description

ImmunoCult™-ACF Human B Cell Expansion Supplement is a serum-free and animal component-free supplement formulated to promote robust in vitro expansion of human peripheral blood B cells in the absence of serum, feeder cells, or specialized culture plates. The supplement contains recombinant proteins and chemically defined components that support the activation and expansion of human B cells and their maturation to plasma cells.

ImmunoCult™-ACF Human B Cell Expansion Supplement is a 50X supplement that can be added to a base medium such as ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) to prepare a complete serum-free medium for culturing and expanding human B cells.

## Properties

**Storage:** Store at -20°C.

**Shelf Life:** Stable for 12 months from date of manufacture (MFG) on label.

**Contains:**

- Recombinant proteins
- Chemically defined components

## Handling / Directions For Use

The following protocol is a guideline and may need to be optimized for the intended application (e.g. by adjusting the cell seeding density or timing of cell density adjustment during cell culture).

### PREPARATION OF B CELLS

Isolate B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs) using one of the following EasySep™ kits:

- EasySep™ Human Pan-B Cell Enrichment Kit (Catalog #19554)
- EasySep™ Human CD19 Positive Selection Kit II (Catalog #17854)
- EasySep™ Human Memory B Cell Isolation Kit (Catalog #17864)

NOTE: This kit can be used to isolate memory and naïve B cell populations from the same sample; both populations can be expanded using ImmunoCult™-ACF Human B Cell Expansion Supplement.

Frozen B cells (e.g. Human Peripheral Blood B Cells, Frozen, Catalog #70023) may also be expanded using ImmunoCult™-ACF Human B Cell Expansion Supplement.

### PREPARATION OF HUMAN B CELL EXPANSION MEDIUM

Use sterile technique to prepare Human B Cell Expansion Medium (ImmunoCult™-ACF Human B Cell Expansion Supplement + base medium). The following example is for preparing 100 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw ImmunoCult™-ACF Human B Cell Expansion Supplement at room temperature (15 - 25°C) until just thawed. If necessary, centrifuge for 30 seconds to recover liquid from the cap. Mix thoroughly.

NOTE: Supplement may appear cloudy; this will not affect performance. If desired, the supplement may be centrifuged briefly or filtered using a 0.2 µm low protein binding filter.

NOTE: If not used immediately, aliquot and store at -20°C. Alternatively, store at 2 - 8°C for up to 4 weeks. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.

2. Add 2 mL of ImmunoCult™-ACF Human B Cell Expansion Supplement to 98 mL of base medium (e.g. ImmunoCult™-XF T Cell Expansion Medium [Catalog #10981]). Mix thoroughly.

NOTE: If not used immediately, store complete medium at 2 - 8°C for up to 2 weeks.

## EXPANSION OF B CELLS

1. Dilute human B cells to  $1 - 2.5 \times 10^5$  cells/mL in complete Human B Cell Expansion Medium and add cell suspension to cultureware as indicated in Table 1.

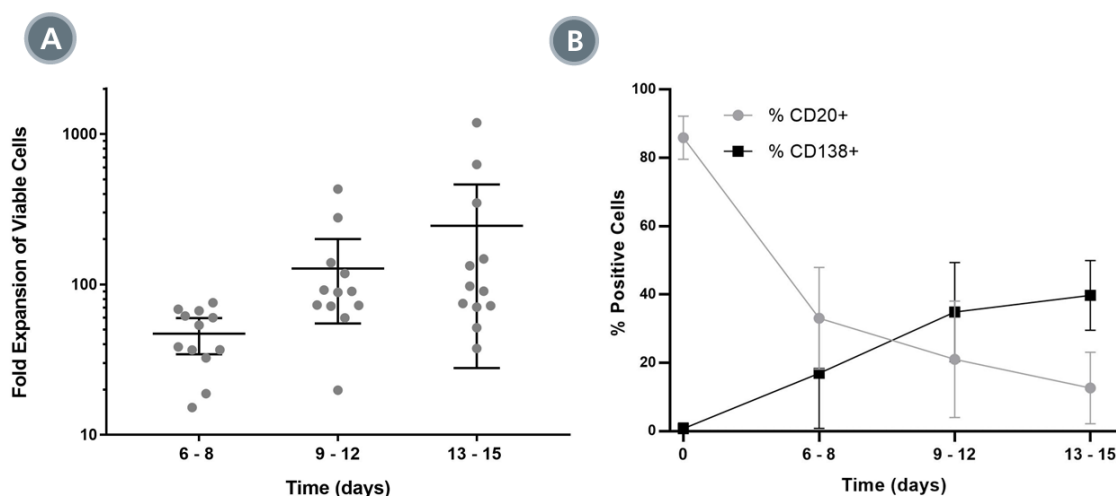
**Table 1. Number of Cells and Volume of Cell Suspension Recommended for Various Cultureware**

| CULTUREWARE   | TOTAL NUMBER OF CELLS (per well) | VOLUME OF CELL SUSPENSION (per well) |
|---------------|----------------------------------|--------------------------------------|
| 6-well plate  | $4 \times 10^5 - 1 \times 10^6$  | 4 mL                                 |
| 12-well plate | $2 - 5 \times 10^5$              | 2 mL                                 |
| 24-well plate | $1 - 2.5 \times 10^5$            | 1 mL                                 |
| 48-well plate | $0.5 - 1.25 \times 10^5$         | 0.5 mL                               |
| 96-well plate | $0.2 - 0.5 \times 10^5$          | 0.2 mL                               |

2. Incubate at 37°C and 5% CO<sub>2</sub> in a humidified incubator.
3. Adjust the cell density to  $1 \times 10^5$  cells/mL every 2 - 3 days as needed by adding fresh Human B Cell Expansion Medium.

NOTE: Expansion is typically accompanied by changes in the expression level of cell surface markers characteristic of B cell activation and maturation, such as downregulation of CD20, upregulation of CD138, and transient upregulation of CD86. These phenotypic changes may be monitored using techniques such as flow cytometry.

## Data

**FIGURE 1. Expansion and Maturation of Human B Cells with ImmunoCult™-ACF Human B Cell Expansion Supplement**

B cells isolated from human PBMCs (leukapheresis packs) using EasySep™ Human Pan-B Cell Enrichment Kit were seeded at  $1 \times 10^5$  cells/well in 24-well tissue culture plates with ImmunoCult™-ACF Human B Cell Expansion Supplement. ImmunoCult™-XF T Cell Expansion Medium was used as a base medium. The cells were passaged every 3 - 4 days.

(A) Fold expansion of viable cells is shown for  $n = 12$  donors, with bars representing the mean and 95% confidence level (range 38- to 1,190-fold at day  $14 \pm 1$  day).

(B) Expression of CD138 and CD20 was analyzed by flow cytometry at each timepoint (data represent % positive viable cells; mean  $\pm$  1 SD). The observed changes indicate maturation of B cells to plasma cells/blasts.

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