

ImmunoCult™ Human B Cell Expansion Kit

Serum-free culture kit for expansion of B cells



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Catalog #100-0645
100-0646
10974

1 Kit
100 mL
2 mL

Product Description

ImmunoCult™ Human B Cell Expansion Kit comprises ImmunoCult™-XF B Cell Base Medium and ImmunoCult™-ACF Human B Cell Expansion Supplement, which together ensure consistent activation and expansion of human B cells and their maturation to plasma cells. B cells can be harvested and used directly in downstream applications after just one week or less in culture.

Product Information

The following products are components of ImmunoCult™ Human B Cell Expansion Kit (Catalog #100-0645) and are also available for individual sale.

PRODUCT NAME	CATALOG #	QUANTITY	STORAGE	SHELF LIFE
ImmunoCult™-XF B Cell Base Medium*	100-0646	100 mL	Store at 2 - 8°C.	Stable until expiry date (EXP) on label.
ImmunoCult™-ACF Human B Cell Expansion Supplement	10974	2 mL	Store at -20°C.	Stable for 12 months from date of manufacture (MFG) on label.

*This product contains material derived from human plasma. Donors have been tested and found negative for hepatitis B surface antigen (HBsAg) and HIV-1 antibodies and/or HIV-1 antigen. However, this product should be considered potentially infectious and treated in accordance with universal handling precautions.

Directions For Use

NOTE: If precipitate is observed in the base medium, centrifuge or filter using a 0.2 - 0.22 µm low protein binding polyethersulfone (PES) filter unit (e.g. Fisher 09-741-04 [0.2 µm, 250 mL]; Fisher SCGP00525 [0.22 µm, 50 mL]). This will not affect performance of the medium.

The following protocol is for the expansion of human B cells using ImmunoCult™-XF B Cell Base Medium supplemented with ImmunoCult™-ACF Human B Cell Expansion Supplement. Depending on the experimental objectives, the protocol may need to be optimized (e.g. cell seeding density or frequency of passaging cells).

PREPARATION OF B CELLS

Isolate B cells from fresh or previously frozen human peripheral blood mononuclear cells (PBMCs), e.g. Human Peripheral Blood Leukopak, Fresh (Catalog #70500) or Frozen (Catalog #70023), 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 EasySep™ 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.

- EasySep™ Human B Cell Isolation Kit (Catalog #17954)
- EasySep™ Direct Human B Cell Isolation Kit (Catalog #19674)
- RosetteSep™ Human B Cell Enrichment Cocktail (Catalog #15024)

NOTE: For more rapid RosetteSep™ processing, this product can be combined with the SepMate™ RUO (Catalog #86450/86415) or SepMate™ IVD* (Catalog #85450/85415) cell isolation tube. For more information on SepMate™, see the associated Product Information Sheets.

*SepMate™ IVD is only available in select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells (MNCs) from whole blood or bone marrow by density gradient centrifugation. In all other regions SepMate™ is available for research use only (RUO).

OR

Thaw frozen B cells (e.g. Human Peripheral Blood B Cells, Frozen [Catalog #70023]).

PREPARATION OF HUMAN B CELL EXPANSION MEDIUM

Use sterile technique to prepare Human B Cell Expansion Medium (ImmunoCult™-XF B Cell Base Medium + ImmunoCult™-ACF Human B Cell Expansion Supplement). 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 ImmunoCult™-XF B Cell Base Medium. 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 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% CO₂ in a humidified incubator.
3. Adjust the cell density to 1×10^5 cells/mL every 2 - 4 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

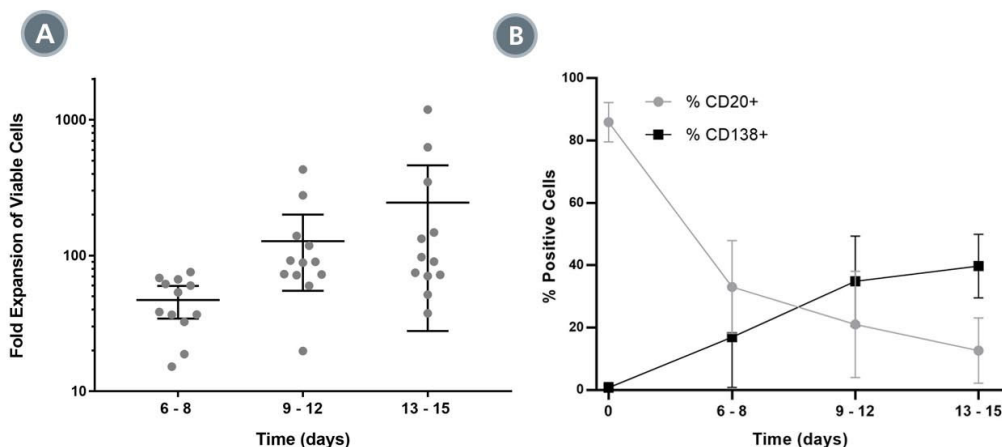


FIGURE 1. Expansion and Maturation of Human B Cells Using Human B Cell Expansion Medium

B cells isolated from human PBMCs (Leukopak) using EasySep™ Human Pan-B Cell Enrichment Kit were seeded at 1×10^5 cells/well in 24-well tissue culture plates with Human B Cell Expansion 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 1190-fold at day 14 ± 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.

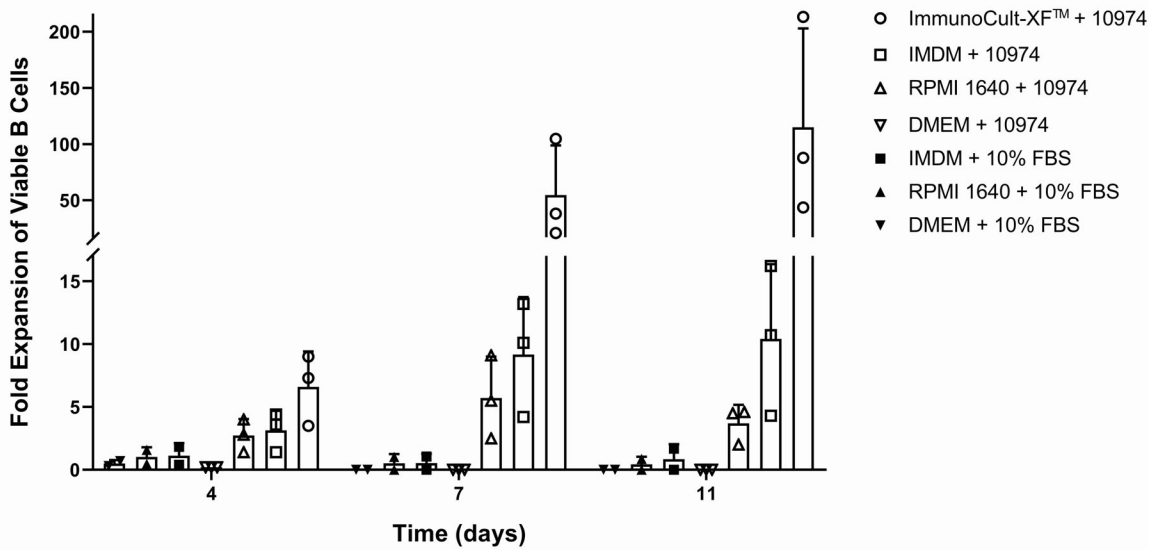


FIGURE 2. Expansion of Human Pan-B Cells Cultured with Various Base Media Supplemented with ImmunoCult™-ACF Human B Cell Expansion Supplement or 10% FBS

Pan-B cells were isolated from human PBMCs (Leukopak) using EasySep™ Human Pan-B Cell Isolation Kit and seeded at 1×10^5 cells/well in 24-well tissue culture plates with ImmunoCult™-XF B Cell Base Medium + ImmunoCult™-ACF Human B Cell Expansion Supplement (Catalog #10974), or other base medium + either ImmunoCult™-ACF Human B Cell Expansion Supplement or 10% fetal bovine serum (FBS). The cells were passaged every 3 - 4 days and the fold expansion of viable cells was calculated at each timepoint. Data represent the mean + 1 SD for $n = 3$ donors (each culture condition was performed in triplicate).

Related Products

For related products, including specialized culture and storage media, supplements, antibodies, cytokines, and small molecules, visit www.stemcell.com or contact us at techsupport@stemcell.com.

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