ImmunoCult™ Mouse B Cell Expansion Kit

Serum-free culture kit for in vitro expansion of mouse B cells

Catalog #100-1003	1 Kit
100-0646	100 mL
100-1004	2 mL

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Product Description

ImmunoCult™ Mouse B Cell Expansion Kit has been developed for the in vitro culture and expansion of mouse splenic B cells. This kit enables the user to expand mouse B cells over 9 days of culture, using ImmunoCult™-XF B Cell Base Medium and ImmunoCult™-ACF Mouse B Cell Expansion Supplement, which together ensure consistent activation and expansion of mouse B cells and their maturation to plasmablasts or plasma cells. The cells can be harvested and used directly in downstream applications after 6 - 9 days in culture.

ImmunoCult™-ACF Mouse B Cell Expansion Supplement is a serum-free and animal component-free (ACF) supplement. It is supplied as a 50X concentrate.

Product Information

The following products are components of ImmunoCult™ Mouse B Cell Expansion Kit (Catalog #100-1003).

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 Mouse B Cell Expansion Supplement	100-1004	2 mL	Store at -20°C.	Stable for 3 years 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.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
D-PBS (Without Ca++ and Mg++)	37350
Tissue culture-treated cultureware	e.g. 38021 (24 wells)
Trypan Blue	07050

Preparation of Reagents and Materials

A. Preparation of B Cells

Isolate B cells from freshly processed mouse splenocytes using EasySep™ Mouse Pan-B Cell Isolation Kit (Catalog #19844).

NOTE: ImmunoCult™ Mouse B Cell Expansion Kit does not support thawed frozen mouse pan-B cells.

B. Preparation of Mouse B Cell Expansion Medium

Use sterile technique to prepare Mouse B Cell Expansion Medium (ImmunoCult™-XF B Cell Base Medium + ImmunoCult™-ACF Mouse B Cell Expansion Supplement). The following example is for preparing 10 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw ImmunoCult™-ACF Mouse 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: If not used immediately, store at 2 - 8°C for up to 4 weeks. Alternatively, aliquot and store at -20°C. Do not exceed the shelf life of the supplement. After thawing aliquots, use immediately. Do not re-freeze.
2. Add 200 µL of ImmunoCult™-ACF Mouse B Cell Expansion Supplement to 9.8 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.

NOTE: Undissolved precipitates can be observed in ImmunoCult™-XF B Cell Base Medium, and undissolved precipitates or cloudiness can be observed in ImmunoCult™-ACF Mouse B Cell Expansion Supplement. Centrifuge or filter the base medium or the supplement 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]) if desired. This will not affect the performance of the kit.

Protocol Diagram

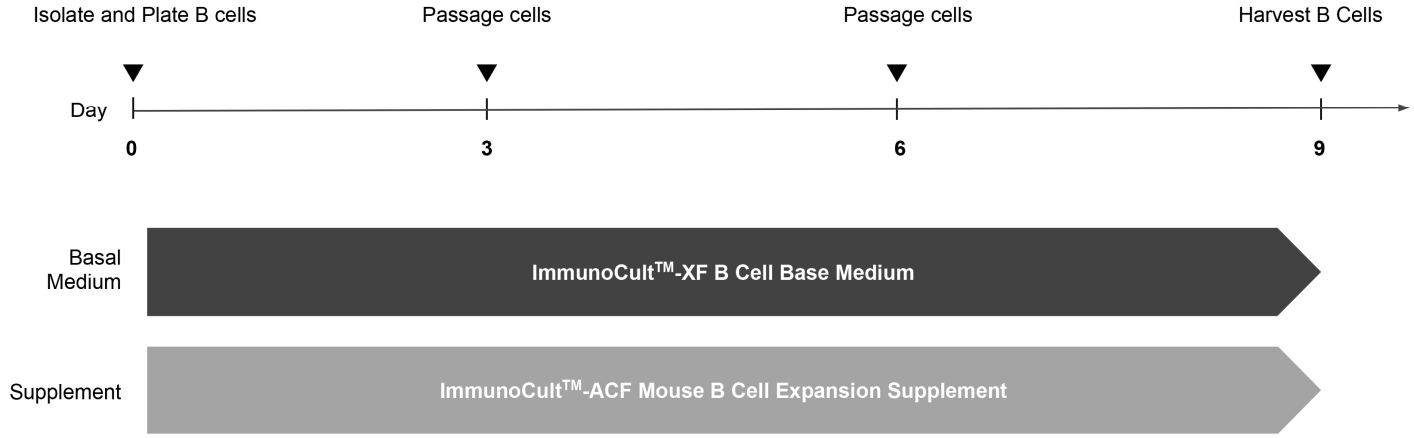

Figure 1. Protocol Diagram for Culturing Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured in complete Mouse B Cell Expansion Medium as described in Directions For Use (steps 1 - 7). B Cells were harvested on day 9 for analysis. Cells can also be harvested at earlier time points depending on different applications.

Directions For Use

Please read the entire protocol before proceeding. For optimal performance, follow the recommended schedule of feeding and passaging; however, the schedule may be adjusted at each step by one day, as long as the cell density does not become over confluent and the medium does not change color. The protocol may need to be optimized (i.e cell seeding density or frequency of passaging cells).

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 x 10^5 - 1 x 10^6	4 mL
12-well plate	2 - 5 x 10^5	2 mL
24-well plate	1 - 2.5 x 10^5	1 mL
48-well plate	0.5 - 1.25 x 10^5	0.5 mL
96-well plate	0.2 - 0.5 x 10^5	0.2 mL

Day 0

1. Isolate B cells as described above and perform a viable cell count using Trypan Blue and a hemocytometer.
2. Dilute mouse B cells to 1 - 2.5 x 10^5 cells/mL in Mouse B Cell Expansion Medium and add cell suspension to the cultureware as indicated in Table 1.
3. Incubate at 37°C and 5% CO_2 in a humidified incubator.

Day 3 and 6: Passage cells

4. Gently pipette up and down in the well to ensure all cells are in suspension.
5. Perform a viable cell count using Trypan Blue and a hemocytometer.
6. Adjust the cell density to 1-2.5 x 10^5 cells/mL every 3 days as needed by transferring a volume of cell suspension containing the recommended number of cells (refer to Table 1) to each new well, and topping up with fresh Mouse B Cell Expansion Medium.
7. Incubate at 37°C and 5% CO_2 in a humidified incubator.

NOTE: Expansion is typically accompanied by changes in the expression level of cell surface markers characteristic of B cell activation and maturation, such as upregulation of CD86 and CD138 respectively (Figure 3). These phenotypic changes may be monitored using techniques such as flow cytometry.

Day 9: Harvest

8. Gently pipette cells up and down to ensure all cells are in suspension. Transfer cells to an appropriate tube. These expanded cells are ready for assays or analysis as required.

Data

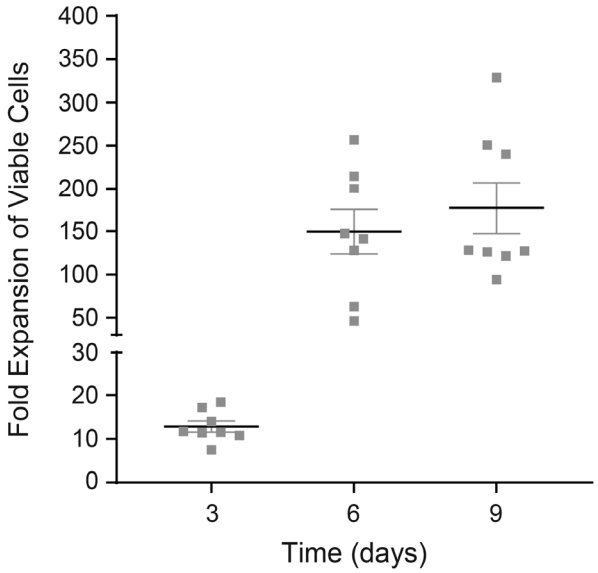

Figure 2. Expansion of Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Figure 1. Fold expansion of viable cells is shown with bar graphs representing the mean ± SEM (n = 8). B cells expanded 176.9 ± 29.8-fold after 9 days of culture.

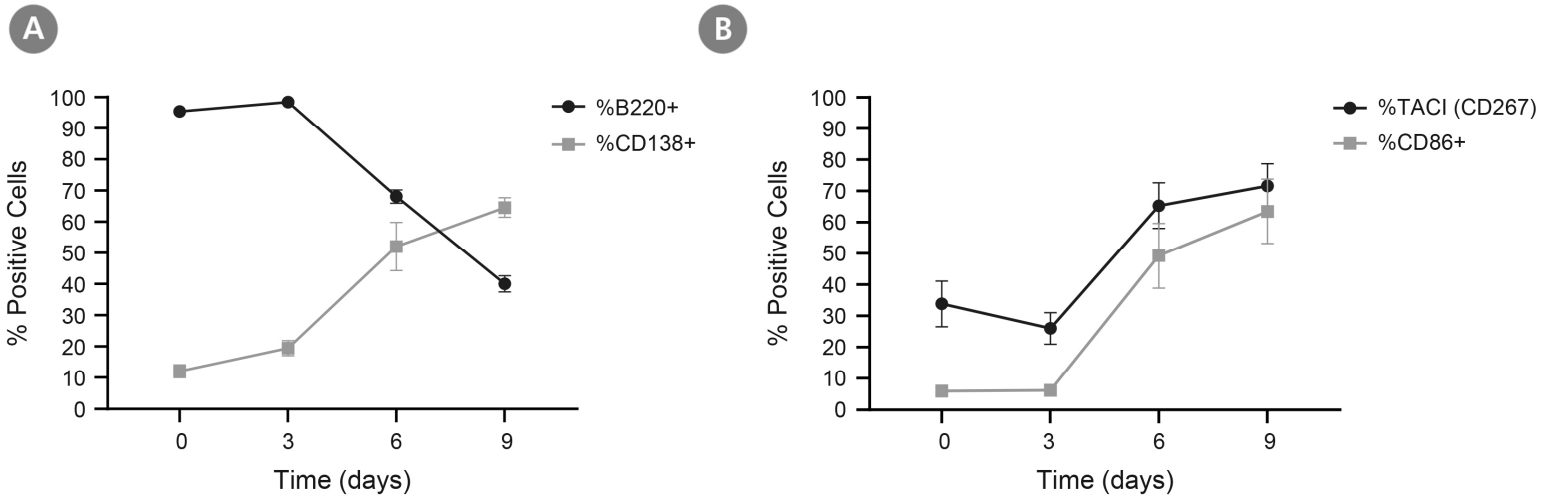

Figure 3. Maturation of Mouse B Cells with ImmunoCult™ Mouse B Cell Expansion Kit

B cells isolated from mouse spleen using EasySep™ Mouse Pan-B Cell Isolation Kit were cultured as described in Figure 1. **A)** Expression of B220 and CD138 and **B)** Expression of TACI (CD267) and CD86 were analyzed by flow cytometry at each timepoint (data represents mean ± SEM, n = 8). An increase in CD86 cell surface expression indicates B cell activation; a decrease in B220 and an increase in CD138 and TACI cell surface expression indicates maturation of B cells to plasmablasts or plasma cells.

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