

PRODUCT DESCRIPTION

B lymphocytes (B cells) play an essential role in the humoral component of the adaptive immune response. Following exposure to a pathogen or foreign material, B cells produce and secrete antibodies that circulate through the body and bind to specific pathogens, marking them for destruction. B cells also contribute to cellular immunity by maintaining immune homeostasis, including the regulation of T lymphocyte activation and expansion. Diseases or abnormalities that affect B cells have been shown to result in immunodeficiencies, autoimmunity, and cancer (1,2). Continued progress in basic and clinical B cell research is crucial for further understanding human health and disease.

ExCellerate B Cell Media, Xeno-Free, is formulated and optimized for the *ex vivo* culture of B lymphocytes. Unlike traditional serum-containing media, ExCellerate B Cell Media provides a stable and optimized culture environment, void of non-human animal-derived products, that facilitates the isolation and expansion of B cells from peripheral blood. This media supports target antigen-specific B cell clonogenicity as well as robust culture of mouse B cell hybridoma cell lines for antibody production. This product does not contain antibiotics.

INTENDED USE

ExCellerate B Cell Media, Xeno-Free, can be used with cytokine/growth factor supplements for the *ex vivo* culture of B cells. This versatile medium is compatible with B cells from multiple species (i.e., human, mouse, rabbit, and canine). The cytokine/growth factor combination used depends upon the experimental design.

STABILITY & STORAGE

Upon receipt, this media should be stored at 2-8 °C until the expiration date on the label.

PRECAUTIONS

The human origin-derived components used in this product have been derived from human plasma, which has been tested at the donor level and found to be negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). All other components are animal-free. However, the media should be handled as if potentially infectious. Safe laboratory procedures should be followed, and protective clothing should be worn when handling this media. The acute and chronic effects of over-exposure to this media are unknown.

When handling bio-hazardous materials such as human cells, safe laboratory procedures should be followed and protective clothing should be worn.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The safety and efficacy of this product in diagnostic or other clinical uses has not been established.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations among primary B lymphocyte populations derived from different donors and species.

PROCEDURE FOR THE *EX VIVO* CULTURE OF HUMAN B LYMPHOCYTES

The protocol below describes the expansion of human B lymphocytes using ExCellerate B Cell Media, in combination with the CellXVivo™ Human B Cell Expansion Kit (R&D Systems, Catalog # CDK005).

Note: *ExCellerate B Cell Media has also been shown to support B cell expansion in protocols using optimized combinations of recombinant cytokines (i.e. IL-4, IL-5, and IL-7). The cytokine/growth factor combinations used with this media should be optimized by application or experimental protocol.*

OTHER MATERIALS REQUIRED

- MagCelect™ Human B Cell Isolation Kit (R&D Systems, Catalog # MAGH103)
- CellXVivo™ B Cell Expansion Kit (R&D Systems, Catalog # CDK005)
- Ficoll-Hypaque™
- Penicillin-Streptomycin
- Resazurin (R&D Systems, Catalog # AR002, or equivalent).
- Pipettes and pipette tips
- Tissue culture flasks and/or plates
- Inverted microscope
- Hemocytometer
- 37 °C, 5% CO₂ incubator
- Centrifuge

B CELL ISOLATION AND EXPANSION

1. Isolate peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
2. Isolate human B cells from PBMCs using the MagCelect™ Human B Cell Isolation Kit. Perform cell count.
3. Add 2 x 10⁵ cells/mL in ExCellerate B Cell Media supplemented with B Cell Expanders 1-3 from the CellXVivo Human B Cell Expansion Kit. Addition of 50 U/mL Penicillin, 50 µg/mL Streptomycin is optional. Suggested plating volumes are shown in Table 1. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 5 days.

Note: *Optimized cytokine combinations may also be used. Protocol optimization may be required.*

Culture Size	Suggested Volume of ExCellerate B Cell Media	B Cell Expander 1-3 Volumes
25 cm ² tissue culture flask	5 mL	10 µL each
75 cm ² tissue culture flask	20 mL	40 µL each
6-well tissue culture plate	3 mL/well	6 µL each/well
24-well tissue culture plate	1 mL/well	2 µL each/well

Table 1: Suggested suspension and plating volumes for B cells.

4. For continued expansion, on day 5 collect B cells by centrifugation at 200 x g for 5 minutes. Resuspend B cells at 2x10⁵ cells/mL in fresh ExCellerate B Cell Media and fresh B Cell Expanders 1-3. Based on the experiment's expansion requirements, re-plate cells using the suggest volumes in Table 1.

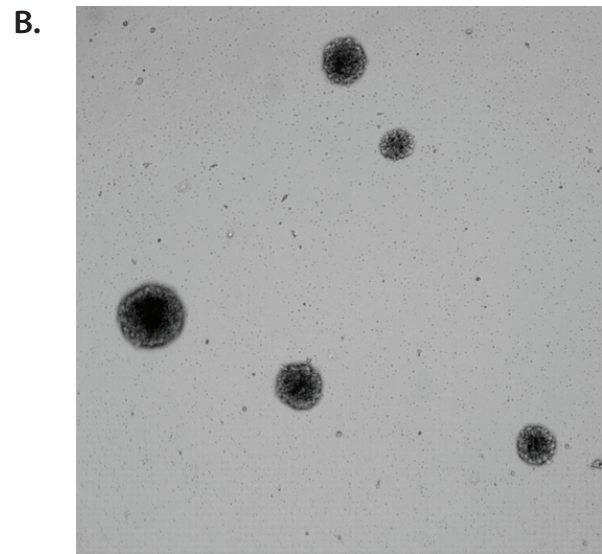
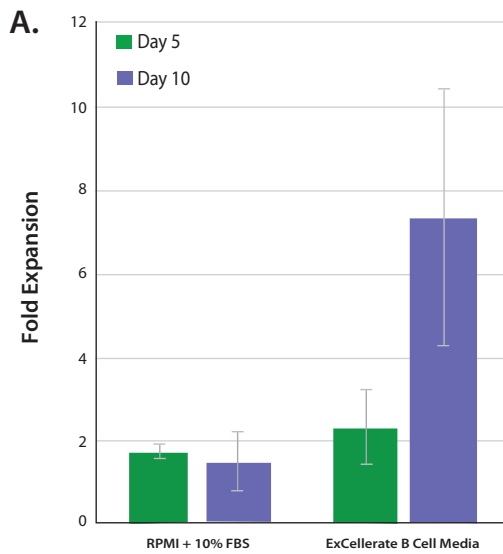
Note: *If using alternative cytokine combinations (i.e. IL-4, IL-5, and IL-7), supplement fresh cytokines into ExCellerate B Cell Media for each expansion passage.*

Optional: *Addition of B Cell Expanders 1-3 (or other cytokine combinations) at Day 3 may improve B cell viability and expansion. We recommend adding B Cell Expanders 1-3 directly to each flask or well using the volumes indicated in Table 1.*

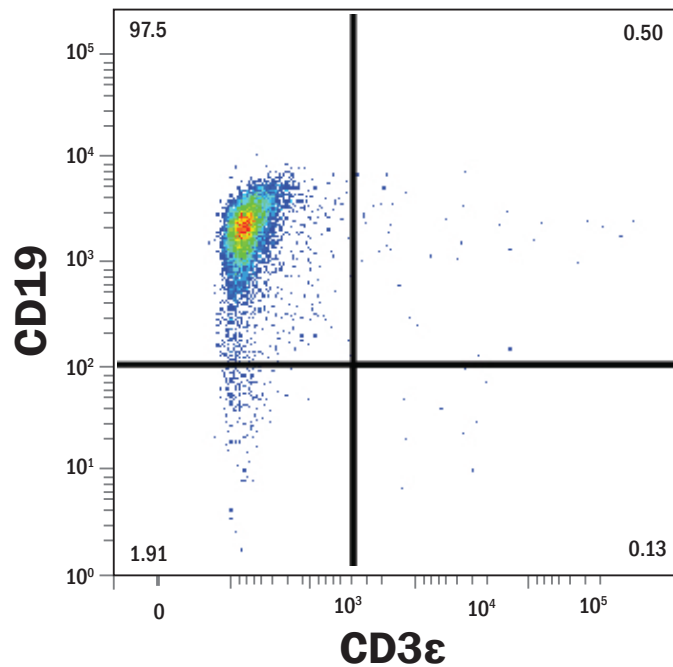
B CELL CHARACTERIZATION

1. Monitor B cell expansion by performing cell counts using a hemacytometer or by using Resazurin.
2. B cell purity can be determined using flow cytometry. B cells will be positive for CD19 (R&D Systems, Catalog # FAB4867) and negative for the T cell marker, CD3 (R&D Systems, Catalog # FAB100A).

DATA EXAMPLES

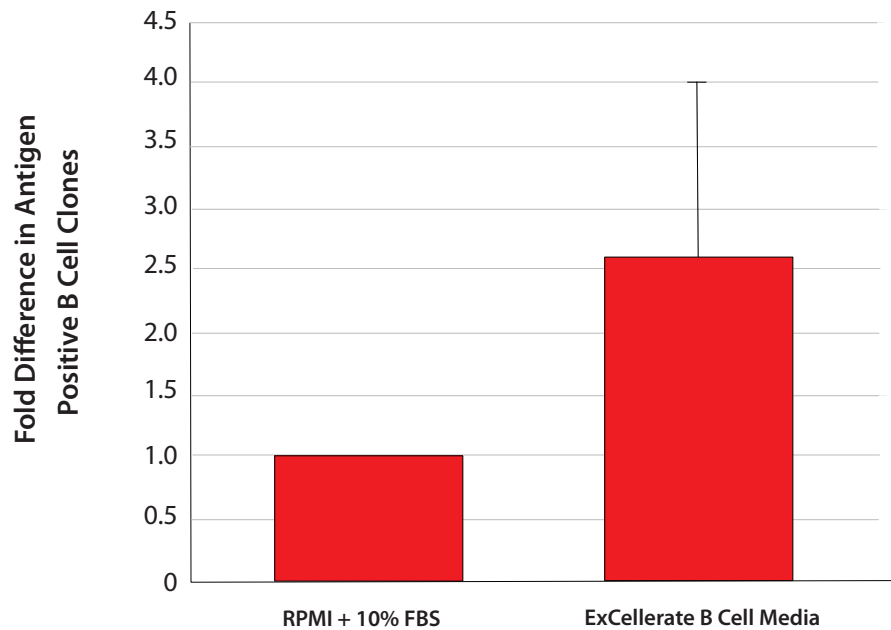


Improved B Cell Expansion using ExCellerate B Cell Media. Human B cells were isolated from PBMCs and cultured for 10 days in either ExCellerate B Cell Media, or RPMI + 10% FBS. Both media conditions were supplemented with reagents included in the CellXVivo Human B Cell Expansion Kit. **A)** Fold expansion of B cells at 5 and 10 days in culture (n=3). **B)** Light microscopy of B cells at 20X magnification after 5 days of culture in ExCellerate B Cell Media.



Detection of CD19 but not CD3 in Human B Cells. Human B Cells were cultured for 5 days in ExCellerate B Cell Media, supplemented with reagents included in the CellXVivo Human B Cell Expansion Kit. The cells were labeled with a Mouse Anti-Human CD19 PE-conjugated Antibody (Catalog # FAB4867P) and with a Mouse Anti-Human CD3 ϵ APC-conjugated (Catalog # FAB100A). CD19⁺CD3⁻ B cells were observed (>97%) following this culture protocol.

DATA EXAMPLES



Improved Positive Clone Isolation of B cells using ExCellerate B Cell Media. Rabbit B cells were isolated from PBMCs and plated into 384-well plates (1 cell/well) using either ExCellerate B Cell Media, or RPMI + 10% FBS. Clonal growth was monitored and screening was performed to identify antigen-specific B cell clones using an ELISA-based protocol and with PCR-based monoclonal cloning of heavy and light chains. Positive identification of clones producing the correct antibody was >2.5 fold higher when cultured using ExCellerate B Cell Media compared to RPMI + 10% FBS (n=8).

REFERENCES

1. LeBien, T.W. and T.F. Tedder. (2008) *Blood* **112**:1570.
2. Basso, K. and R. Dalla-Favera (2015) *Nat. Rev. Immunol.* **15**:172.