

DATA EXAMPLES

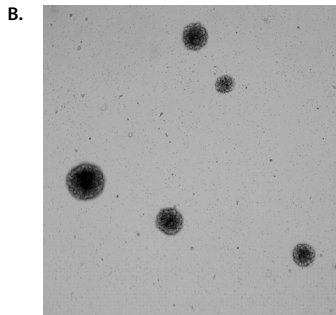
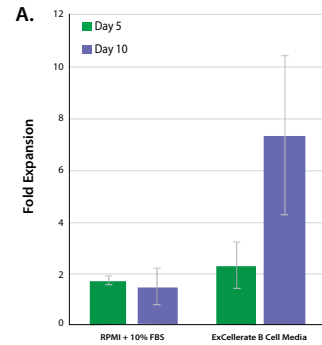


Figure 1: Improved B Cell Expansion using ExCellerate B Cell Media. Human B cells were isolated from PBMCs and cultured for 10 days in either ExCellerate B Cell Media, or RPMI + 10% FBS. Both media conditions were supplemented with reagents included in the CellXVivo Human B Cell Expansion Kit. **A)** 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.

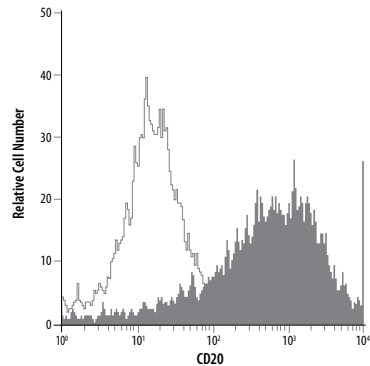


Figure 2: Detection of CD20 in Human B Cells. Human B cells were expanded for 5 days in RPMI base media and using reagents included in the Human B Cell Expansion Kit. The cells were labeled with a PE-conjugated Mouse Anti-Human CD20 Monoclonal Antibody (R&D Systems, Catalog # FAB4225P; filled histogram) or a PE-conjugated Mouse IgG1 Isotype control (R&D Systems, Catalog # IC002P; open histogram).

DATA EXAMPLES CONTINUED

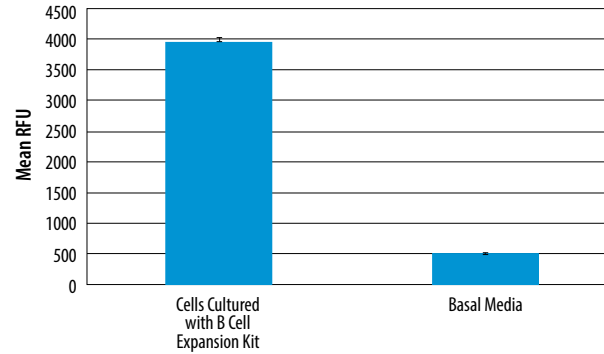


Figure 3: Human B Cell Expansion. Human B cells were isolated from PMBCs and cultured for 5 days in RPMI base media and using reagents included in the Human B Cell Expansion Kit. B cell expansion was measured with Resazurin (R&D Systems, Catalog # AR002).

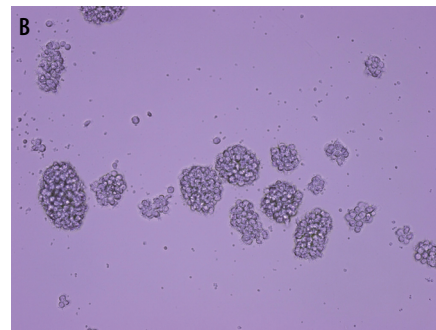


Figure 4: Light Microscopy of B Cells. (A) B cells were imaged at 20X magnification following isolation from PMBCs and (B) after 5 days of culture in RPMI base media and using reagents included in the Human B Cell Expansion Kit.

CellXVivo™

Human B Cell Expansion Kit

Catalog Number: CDK005

BACKGROUND

B cells are a subset of lymphocytes that express immunoglobulin receptors which are tailored for specific antigens (1, 2). The B cell population is a vital component of the humoral immune response. B cells are involved in antigen presentation, cytokine homeostasis, and antibody production (3). The Human B Cell Expansion Kit contains the necessary components to induce human B cell proliferation. The kit contains sufficient reagents to expand a starting population of 10⁷ human B cells 3-5 fold.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956 FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China

Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475 FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

COMPONENTS	PART #	# VIALS	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
B Cell Expander 1	967572	1 vial	Store at 2-8 °C under sterile conditions for up to 30 days or at -20 °C to -70 °C in a manual defrost freezer for up to 3 months.*
B Cell Expander 2	967573	1 vial	
B Cell Expander 3	967574	1 vial	
Reconstitution Buffer 1	967552	1 vial	Store at 2-8 °C under sterile conditions for up to 3 months.*
Reconstitution Buffer 2	967553	1 vial	

* Provided this is within the expiration date of the kit.

OTHER MATERIALS & SUPPLIES REQUIRED

- Ficoll-Hypaque™
- MagCelect™ Human B Cell Isolation Kit (R&D Systems, Catalog # MAGH103, or equivalent)
- ExCellerate B Cell Media (R&D Systems, Catalog # CCM031)
- RPMI 1640
- Fetal Bovine Serum (FBS)
- L-Glutamine/Penicillin/Streptomycin (optional)
- β-Mercaptoethanol (2-ME)
- 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

REAGENT PREPARATION

B Cell Expander 1 (500X) - Add 110 μL of Reconstitution Buffer 1 to B Cell Expander 1 to produce B Cell Expander 1 (500X).

B Cell Expander 2 (500X) - Add 110 μL of Reconstitution Buffer 1 to B Cell Expander 2 to produce B Cell Expander 2 (500X).

B Cell Expander 3 (500X) - Add 110 μL of Reconstitution Buffer 2 to B Cell Expander 3 to produce B Cell Expander 3 (500X).

PROTOCOL FOR B CELL EXPANSION

1. Isolate human peripheral blood mononuclear cells (PBMCs) from human blood using Ficoll-Hypaque density gradient centrifugation.
2. Isolate human B cells from the PBMCs using the MagCelect Human B Cell Isolation Kit.
3. Suspend 2×10^5 human B cells/mL in base media containing B Cell Expanders 1-3 as suggested in Table 1.

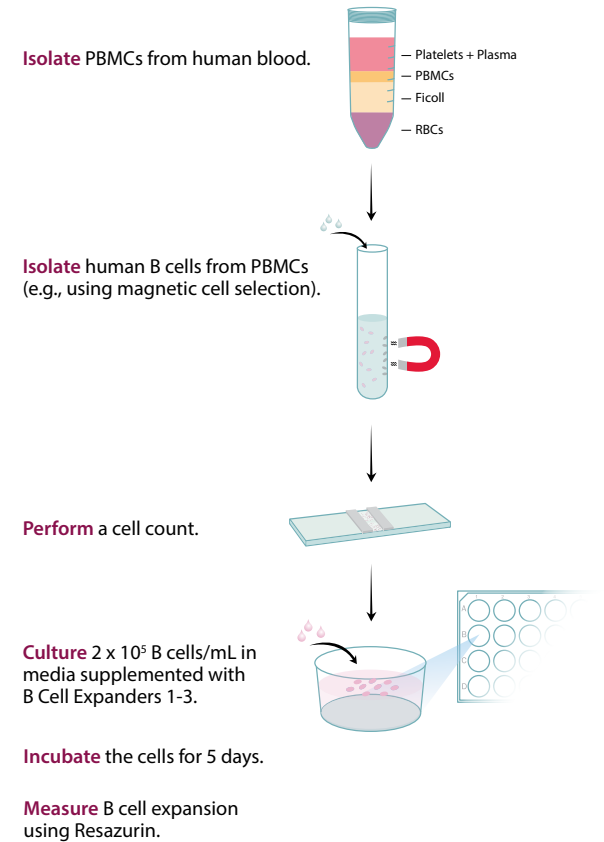
Note: For base media we recommend using ExCellerate B Cell Media or RPMI 1640 supplemented with 10% FBS, 2 mM L-Glutamine, 50 μg/mL penicillin, 50 μg/mL Streptomycin, 50 μM β-mercaptoethanol.

Size	Suggested Culture Volume	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 culture volumes for media and expanders.

4. Incubate the cells in a 37 °C, 5% CO₂ humidified incubator for 5 days.
5. Measure human B cell expansion using Resazurin.

PROTOCOL OUTLINE



REFERENCES

1. Spriggs, M. *et al.* (1992) J. Exp. Med. **176**:1543.
2. Pietravalle, F. *et al.* (1996) J. Biol. Chem. **271**:5965.
3. LeBien, T. *et al.* (2008) Blood **112**:1570.