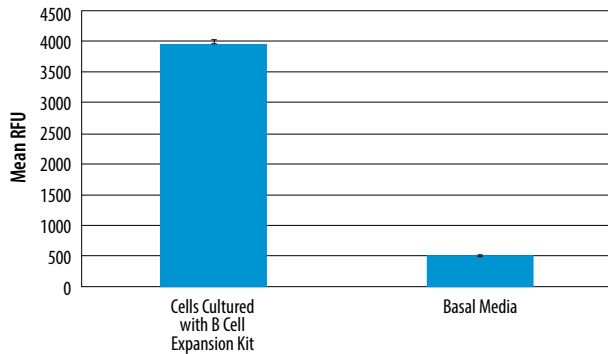
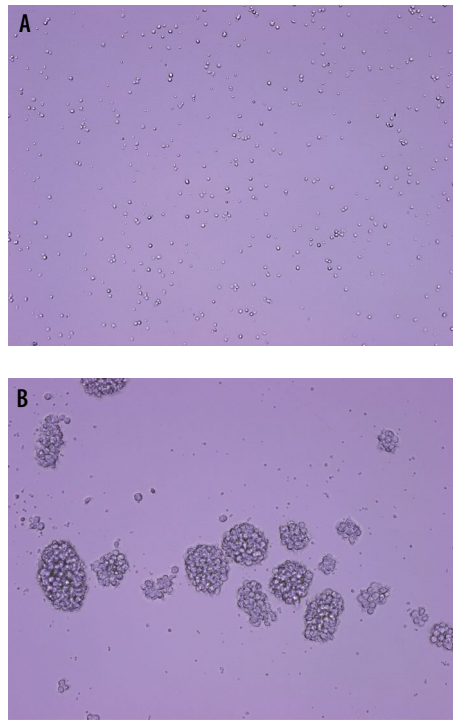


**Figure 1: Detection of CD20 in Human B Cells.** Human B cells were expanded for 5 days using reagents included in the Human B Cell Expansion Kit. The cells were labeled with a PE-conjugated Mouse Anti-Human CD20 Monoclonal Antibody (R&D Systems, Catalog # FAB4225P; filled histogram) or a PE-conjugated Mouse IgG1 isotype control (R&D Systems, Catalog # IC002P; open histogram).



**Figure 2: Human B Cell Expansion.** Human B cells were isolated from PMBCs and cultured for 5 days using reagents included in the Human B Cell Expansion Kit. B cell expansion was measured with Resazurin (R&D Systems, Catalog # AR002).



**Figure 3: Light Microscopy of B Cells.** (A) B cells were imaged at 20X magnification following isolation from PMBCs and (B) after 5 days in culture using reagents included in the Human B Cell Expansion Kit.

**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.

**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 10<sup>7</sup> 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 & Canada | R&D Systems, Inc.**  
 614 McKinley Place NE, Minneapolis, MN 55413, USA  
 TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400  
 E-MAIL: info@RnDSystems.com

**DISTRIBUTED BY:**

**UK & Europe | R&D Systems Europe, Ltd.**  
 19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK  
 TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420  
 E-MAIL: info@RnDSystems.co.uk

**China | R&D Systems China Co., Ltd.**

24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050  
 TEL: +86 (21) 52380373 FAX: +86 (21) 52371001  
 E-MAIL: info@RnDSystemsChina.com.cn

## 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

- MagCelect™ Human B Cell Isolation Kit (R&D Systems, Catalog # MAGH103, or equivalent).
- Resazurin (R&D Systems, Catalog # AR002, or equivalent).
- Ficoll-Hypaque™
- RPMI 1640
- L-Glutamine
- Penicillin
- Streptomycin
- Fetal Bovine Serum (FBS)
- β-Mercaptoethanol
- Tissue culture flasks and/or plates
- Inverted microscope
- Hemocytometer
- 37 °C and 5% CO<sub>2</sub> humidified cell culture incubator
- Benchtop centrifuge
- Pipettes and pipette tips

## 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. Add  $2 \times 10^5$  human B cells/mL in RPMI 1640 supplemented with 10% FBS, 2 mM L-Glutamine, 50 U/mL penicillin, 50 µg/mL streptomycin, 50 µM β-mercaptoethanol, and B Cell Expanders 1-3 as suggested below:

Size	Suggested Culture Volume	B Cell Expander 1-3 Volumes
25 cm <sup>2</sup> tissue culture flask	5 mL	10 µL each
75 cm <sup>2</sup> 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

4. Incubate the cells in a 37 °C, 5% CO<sub>2</sub> humidified incubator for 5 days.
5. Measure human B cell expansion using Resazurin.

## PROTOCOL OUTLINE

