Human B Cell Culture and Expansion Kit

1. Introduction

Human B cell culture and expansion kit is for activation and expansion of human B cells. Human monoclonal antibodies isolated for specific antibodies are widely used in immunity studies, historical studies and therapeutic development. Memory B cells derived from peripheral blood will produce high concentration of IgG in the supernatant under the stimulation of CD40L with other growth factors. The B Cell Expansion Kit contains special treated feeder cells, expressing CD40L in activated form as well as human cell factors A and B potently inducing antibody-secret. The monoclonal antibody screening can be completed within 2 weeks with the help of the kit.

2. Product Information

Catalog No.	Product name	Size
BC1001	Human B Cell Culture and Expansion Kit	1 package

3. Guidelines for using Human B Cell Culture and Expansion Kit

General

A. Human B cells isolation from PBMC.

Procedure

B. Culture human B cells in presence of inactive feeder cells and cell factor A, B and feeder cells for 13 days.

C. Screening B cell supernatants for functional assay.

Preparation of plating setup

1. Thaw total of 7 × 10^6 feeder cells, and resuspend the cells in 1.5 ml of pre warmed IMDM medium containing 15 µl of benzonase.
2. Centrifuge the cell suspensions at 335g for 10 min at 4 °C. Resuspend the resulting cell pellet in 7 ml of IMDM medium.
3. Calculate the reagent amounts. For 4 plates, an arrangement that corresponds to an overall feeder mixture volume of 70 ml, the composition of the mixture is described as below.
4. Prepare the feeder mixture as calculated in Step 5 to achieve a confluent, single-cell layer of 5,000 fibroblasts per well.
5. Set aside 5 ml of the mixture (corresponding to 4 plates) to be used as a ‘no–B cell’ (non-antibody) control.
6. For 4 plates, the composition of the culture mixture is described in the following table:

Component	Volume
Complete IMDM medium	60 ml
Human cell factor A solution	800 ul
Human cell factor B solution	400 µl
Feeder cells (step 2)	7 ml

Plating B cells

7. Label the lids and sides of each plate with numbers and the sort date. Use 12-channel pipettes to plate the cells. Angled plate holders may be useful, if the cells are plated with a pipette.
8. Add 50 µl per well of sterile water to the outer wells (wells A1-A24, P1-P24, B1-O1 and B24-O24; ~7.7 ml of sterile water are needed per plate), in order to diminish evaporation from the inner culture wells.
9. Before adding B cells to the feeder mixture, plate 50 µl per well of the culture mixture set aside in Step 6 for the non-antibody control in each plate. The culture supernatants of non-antibody control will be collected after 13 d and used as no-IgG controls in screening and compared with the supernatants from other rows.
10. Sort single B cells, and the cell purity should be above 90%.
11. Calculate the number of cells needed to plate at the desired density, between 1.3 and 4 B cells per well, depending on the desired result. As an example, 4 plates at a density of 4 B cells per well is equal to 4 cells per well × 308 wells per plate × 4 plates = 4,928 B cells.
12. Add an appropriate amount of B cells into 2ml complete IMDM medium as calculated in Step 6 to the culture mixture. Move the cell mixture to large sterile basins, and plate 50 µl per well in the inner 308 wells (except non-antibody control). Ensure that you gently mix the cells in the basin frequently to keep the B cells evenly suspended.
13. Move the 384-well tissue culture plates to a humidified 5% CO2 incubator set to 37 °C.

B cell culture

14. Leave the plates undisturbed in the incubator for 13–14 d. incubating the plates for less than 12 d may result in insufficient accumulation of IgG in the supernatants. In contrast, after 15 d, the cultures are expected to die off.
15. Inspect during culturing:

Day 10: visual inspection of plates. If you are aiming for the high seeding density of four B cells per well, it may be possible to visualize the expanding B cells in many wells on days 10 and 11. They will be small and refractile. All wells will contain debris from feeder cells, which begin to die on day 3.

Day 12: measurement of IgG concentration in supernatants from a quarter of the wells from one plate by ELISA.

Supernatant collection and B cell lysis

16. Use 12-channel pipettes to move 35 µl of supernatant from each well of the old plates to a corresponding well on the pre-labeled new plates. A suitable approach to this task is to touch the bottom lightly, move the tips up slightly and then pull up the supernatants slowly to avoid aspirating B cells before dispensing into new plates.
17. Cover the plates to which the supernatants have been added with aluminized foil seals and put the lids on. Store the supernatant plates at −80 °C for an appropriate functional screening. The supernatant plates can be stored at −80 °C for up to 6 months.
18. Add 20 µl of lysis buffer to all wells containing B cells. Cover the plates with aluminized foil seals and put the lids on. Immediately store the B cell lysis plates at −80 °C for future use in Ig gene amplification and cloning. The B cell lysis plates can be stored at −80 °C for up to 2 years.

Troubleshooting

1. *Cell contamination*

   Filter the solution and medium before the assay. Thoroughly clean the hood and pipettes with 70% (vol/vol) ethanol. Careful operation is needed through the procedure.

2. *Low percentage of IgG-positive wells*

   Try to plate B cells as soon as possible after sorting to increase the viability of B cells. Sort and aliquot as many B cells as possible for culture. Adding dditional fluorochrome-conjugated antibodies for T cells, monocytes, natural killer cells and macrophages is helpful to sort B cells correctly. Use an automated cell counter.

3. *Isolated antibodies are not potent*

   Screen more B cells. Test different viruses for neutralization screening.

   Screen the patient serum with viruses from different clades and select the patient with potent and broad neutralizing activity.

4. *False positive IgG sequencing results*

   Make sure the hood is clean to avoid both bacterial and DNA contamination. Do not Use a hood that is also used for IgG transfections, as this practice may result in PCR contamination and in amplification of contaminating plasmid sequences in the following steps.

Components

Special treated feeder cells, 7 x 10^6

Human cell factor A solution, premium grade

Human cell factor B solution, premium grade

For 4 384-well tissue culture plates.

Reference

J. Huang, N. A. Doria-Rose, N. S. Longo, L. Laub, C.-L. Lin, E. Turk, B. H. Kang, S. A. Migueles, R. T. Bailer, J. R. Mascola, M. Connors, Isolation of Human Monoclonal Antibodies from Peripheral Blood B Cells. Nat. Protoc. 8, 1907-1915 (2013).

Note

For laboratory research only. Not for clinical applications.