Miltenyi Biotec

B Cell Expansion Kits
human

B Cell Expansion Kit, human	130-106-196
B Cell Expansion Kit, human – small size	130-124-195

Contents

1. Description

This product is for research use only.

Components

1000 µg Human CD40-Ligand Multimer Kit: Recombinant human CD40-Ligand, premium grade (2×500 µg) and Cross-Linking Antibody (2×1 mL).

5 µg Human IL-4, premium grade

500 mL StemMACS™ HSC Expansion Medium XF, human

or

200 µg Human CD40-Ligand Multimer Kit: Recombinant human CD40-Ligand, premium grade (2×100 µg) and Cross-Linking Antibody (2×0.2 mL).

5 µg Human IL-4, premium grade

100 mL StemMACS™ HSC Expansion Medium XF, human

Biological activity

CD40-Ligand:
The ED_{50} of multimerized Human CD40-Ligand is ≤250 ng/mL corresponding to a specific activity of ≥4×10^3 U/mg. The biological activity of the specific cytokine lot is indicated on the Certificate of Analysis (https://www.miltenyibiotec.com/certificates).

▲ **Note:** The ED_{50} of multimerized Human CD40-Ligand is determined by proliferation assay using enriched $CD19^+$ B cells in the presence of the cross-linking antibody and 50 IU/mL interleukin 4 according to Spriggs, M.K. *et al.*[1] Activity was also shown by activation assay using enriched $CD19^+$ B cells in the presence of 50 IU/mL interleukin 4.

IL-4:
The ED_{50} is ≤0.2 ng/mL corresponding to an activity of ≥5×10^6 IU/mg. The biological activity of the specific cytokine lot is indicated on the Certificate of Analysis (https://www.miltenyibiotec.com/certificates).

▲ **Note:** The ED_{50} is determined by proliferation assay using TF-1 cells according to Kitamura *et al.*[1] The proliferation assay was calibrated with the international standard for human IL-4 (NIBSC code 88/656) provided by the WHO/National Institute for Biological Standards and Control.

Product format

All components are supplied in azide-free buffer containing stabilizer. Low endotoxin.

Storage

The Cross-Linking Antibody should be stored at 2–8 °C. Lyophilized reagents and medium should be stored at –20 °C. The expiration date is indicated on the vial label. For information about reconstitution of the lyophilized reagents and storage after reconstitution refer to chapter 2.1.

1.1 Principle of the B Cell Expansion Kit

The B Cell Expansion Kits have been developed for the activation and expansion of human B cells. The CD40-Ligand Multimer mimics T cell–dependent activation of B cells. The B cell expansion is achieved by culturing and restimulation at day 7 and 10 of culture.

1.2 Applications

- Activation and expansion of human B cells.

1.3 Reagent and instrument requirements

- CD19 MicroBeads, human (# 130-050-301)
- AB serum
- Humidified incubator
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, for example, CD80-PE, CD86-FITC, and CD19-APC. For more information about fluorochrome-conjugated antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for the flow cytometric exclusion of dead cells.

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2. Protocol

▲ All steps in the protocol have to be performed under sterile conditions.

2.1 Reconstitution of lyophilized reagents

It is recommended to reconstitute lyophilized Human CD40-Ligand with deionized sterile filtered water to a final concentration of 0.5 mg/mL in a volume of 200 µL (for 100 µg size) or 1000 µL (for 500 µg size) and store at –20 °C.
To increase the biological activity, we highly recommend multimerization of Human CD40-Ligand and Cross-Linking Antibody. Therefore, equal volumes of both components (e.g. 40 µL of the reconstituted CD40-Ligand and 40 µL of the Cross-Linking Antibody) should be incubated together for 30 minutes at room temperature (19–25 °C) prior to media application. Reconstitute Human IL-4 with deionized sterile-filtered water to a final concentration of 2.5×10^5 IU/mL, e.g. if the lot has a biological activity of 8×10^6 IU/mg, 160 µL water should be used for reconstitution of 5 µg. Mix by pipetting up and down until resuspended. To avoid repeated freeze-thaw cycles, reconstituted reagents should be aliquoted and stored at –20 °C or below until use.

2.2 B cell isolation

Isolate B cells using CD19 MicroBeads, human (# 130-050-301). For details refer to the respective data sheet.

2.3 B cell expansion

This protocol is optimized for the activation and expansion of B cells, using the Human CD40-Ligand Multimer Kit. B cells can either be expanded in low density (0.15×10^6 cells/mL) or in high density (1×10^6 cells/mL) cell culture (refer to table in section 4). The low density protocol will increase B cell expansion by up to 10-fold.

▲ Volumes given below are for 10 mL of final expansion medium. When working with larger volumes please scale up accordingly.

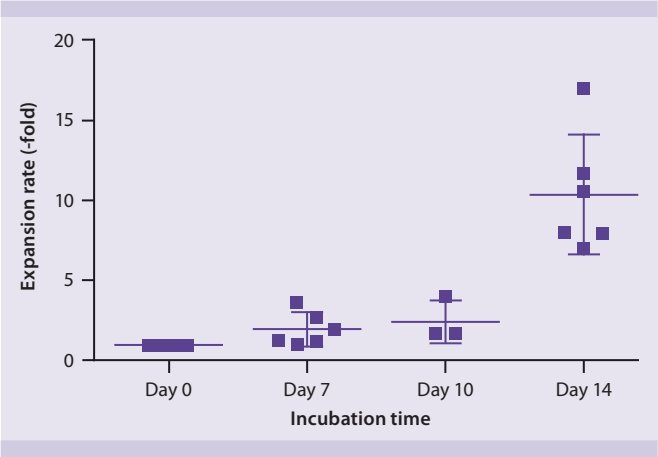
▲ The final expansion medium should be prepared directly before the stimulation. Long-term storage is not recommended.

1. Prepare 10 mL StemMACS™ HSC Expansion Media XF with 2 µL reconstituted Human IL-4, and 5% AB serum.
2. Pre-incubation of the components of the CD40-Ligand Multimer Kit: Mix 40 µL reconstituted Human CD40-Ligand with 40 µL Cross-Linking Antibody. Incubate for 30 minutes at room temperature. Subsequently, add the multimerized Human CD40-Ligand (80 µL) to 10 mL prepared StemMACS HSC Expansion Media XF (final expansion medium).
3. Resuspend B cells at a density of 10^6 cells (high density) or 0.15×10^6 cells (low density) in 1 mL of the final expansion medium and culture in suitable cell culture plate. For details refer to chapter 4. Appendix: Culture plate sizes for B cell expansion.
4. Incubate at 37 °C, 5% CO_2.
   ▲ **Note:** In high density culture exchange half of the medium on day 5 with fresh medium supplemented with 5% AB serum and 50 units IL-4/mL (0.2 µL IL-4 per mL media).
5. On day 7 and 10 harvest B cells and repeat step 1–4.
6. On day 14 harvest B cells and proceed to downstream applications.

3. Examples of B cell expansion using the B Cell Expansion Kit

For the expansion of B cells using the B Cell Expansion Kits 80 µL multimerized Human CD40-Ligand are used per 10 mL prepared StemMACS HSC Expansion Media XF. Please note, that optimal concentration and ratio will depend on the application as well as the used assay.

The components of the CD40-Ligand Multimer Kit were pre-incubated for 30 minutes at room temperature (as described in 2.4.2). B cells were expanded with a starting density of 0.15×10^6 cells/mL (low density) according to the protocol, as described in section 2.4. On day 7 and 10 cells were harvested, counted, and restimulated. All cells were harvested, counted, and analyzed on day 14. With the low density protocol a B cell expansion of up to 10-fold was achieved.



4. Appendix: Culture plate sizes for B cell expansion

For B cell expansion the cells should be resuspended in culture medium at 1×10^6 cells/mL or 0.15×10^6 cells/mL.
The following table lists culture plate sizes suitable for different cell numbers. It also indicates the appropriate amount of medium to be added.

Total cell number (high density)	Total cell number (low density)	Final medium volume	Culture plate
0.5×10^6	0.075×10^6	0.5 mL	48 well
1.0×10^6	0.15×10^6	1 mL	24 well
2.0×10^6	0.3×10^6	2 mL	12 well
4.0×10^6	0.6×10^6	4 mL	6 well

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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