CD28/CD3 T CELL ACTIVATION/EXPANSION BEADS

OPTIMIZED FOR CAR-T MANUFACTURING

AMS.PM-CAR2002-2ML
-2002-10ML

DESCRIPTION

The purpose of this product is to activate and expand human T cells: CD4+, CD8+ T cells, antigen specific T cells or polyclonal T cells using Promab's CD3/CD28 Macrobeads™.

MATERIALS AND PRINCIPLE OF T CELL ACTIVATION/EXPANSION KIT

Macrobeads™ Human T-Activator CD3/CD28 are uniform 3.0 μm magnetic polymer beads coated with an optimized mixture of monoclonal antibodies against the CD3 and CD28 cell surface molecules of human T cells (*Figure 1*). The CD3 antibody is specific for the epsilon chain of human CD3, which is a subunit of the TCR complex. The CD28 antibody is specific for the human CD28 co-stimulatory molecule, which is the receptor for CD80 (B7-1) and CD86 (B7-2). Both antibodies are mouse anti-human IgGs coupled to the same bead, mimicking in vivo stimulation by Antigen Presenting Cells (Dendritic Cells) (*Figure 1*). Both the bead size and the covalent antibody coupling technology are critical parameters to allow the simultaneous presentation of optimal stimulatory signals to the T cells in culture, thus allowing their full activation and expansion.

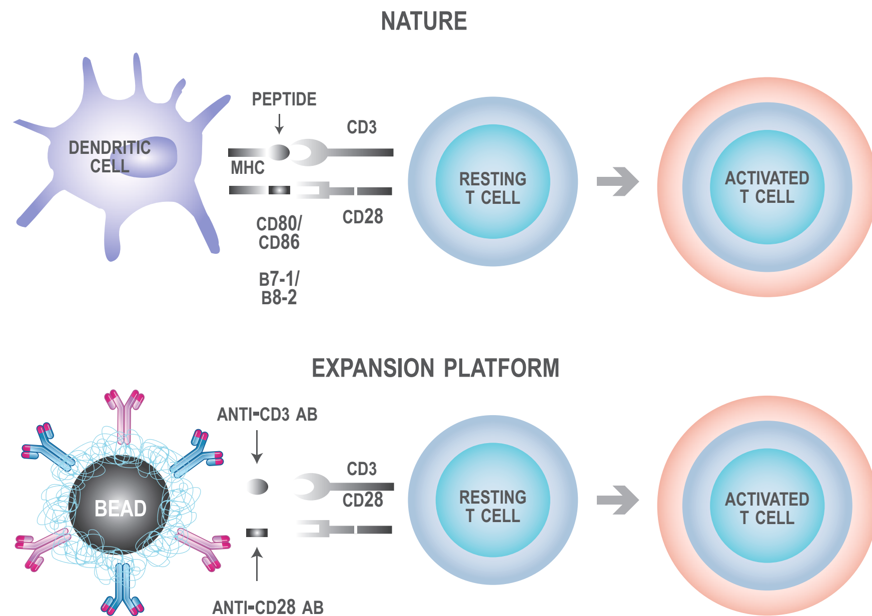
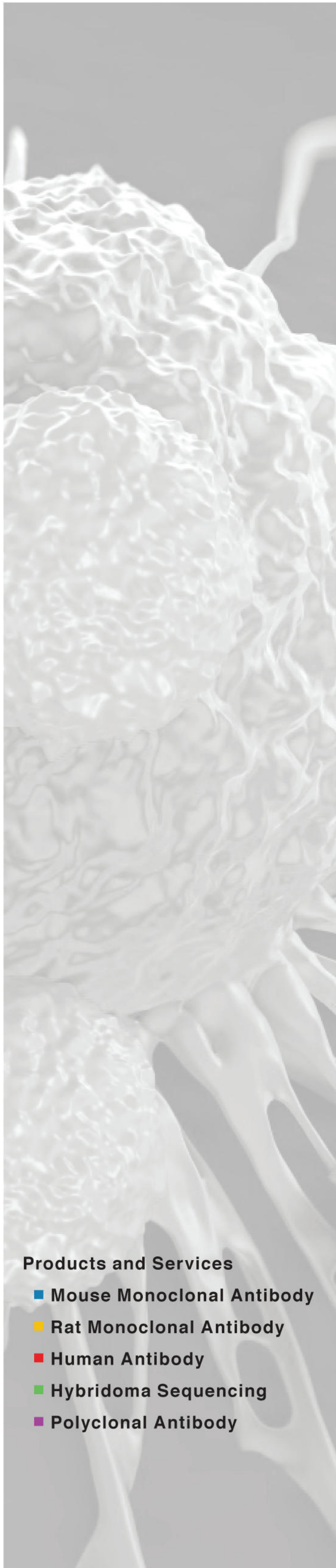


Figure 1. Activation of T cells in Nature and in Expansion Platforms



APPLICATIONS

The activated T cells can be analyzed after activation (for transfection/ transduction or for other biochemical assays: genomics, proteomics, and T cell functional immunology assays). T cells can be cultured to differentiate into T helper cell subsets, T cell proliferation/expansion of Ag-specific or polyclonal T cells.

ADDITIONAL MATERIALS REQUIRED

1. Buffer:
   Phosphate buffered saline with 0.1% bovine serum albumin and 2 mM EDTA, pH 7.4 (PBS w/0.1% BSA).
2. Centrifuge.
3. Culture medium:
   Advanced RPMI Medium 1640 with 2 mM L-Glutamin, 10% FCS/ FBS and 100 U/ml penicillin/streptomycin or OpTmizer™ T Cell Expansion SFM with 100 U/ml penicillin/streptomycin, or another equivalent culture medium.
4. Recombinant human IL-2.
5. Heat inactivated Fetal Calf Serum (FCS).
6. Flat bottom tissue culture plates or tissue culture flasks.
7. Humidified CO_2 incubator.

RECOMMENDATIONS

1. Resuspend the Macrobeads™ in the vial carefully before use, i.e. vortex for >30 sec., or rotate for 5 minutes.
2. Never use less than the recommended volume of Macrobeads™.
3. Carefully follow the recommended pipetting volumes.
4. Avoid air bubbles during pipetting.
5. Prior to flow cytometric analysis, Macrobeads™ and bead-bound cells should be removed. Upon activation and for 2-3 days thereafter, some cells will bind strongly to the beads. Resuspend the bead/cell suspen sion thoroughly by pipetting to increase cell recovery, separate on a magnet (after transfer to a suitable tube) and collect supernatant con taining the T cells. The bead-bound cell fraction can be cultured over night and the above process repeated to increase T cell recovery. When using cells for proteomics or genomics studies, lyse the cells prior to bead removal.
6. Follow the procedure described in the respective package insert.
7. Prepare cell culture medium.

WASHING OF MACROBEADSTM BEFORE USE

Washing of Macrobeads™ before use

1. Resuspend the MacrobeadsTM in the vial (i.e. vortex for >30 sec, or tilt and rotate for 5 min).
2. Transfer the desired volume of Macrobeads™ to a tube.
3. Add an equal volume of buffer, or at least 1 mL, and mix (vortex for 5 sec, or keep on a roller for at least 5 min).
4. Centriguge at 300xg for 1 min.
5. Aspirate supernatant completely. Resuspend the washed Macrobeads™ in thesame volume of culture medium as the initial volume of Macrobeads™ taken from the vial.

ACTIVATE HUMAN T CELLS

1. Start with 1×10^6 purified T cells in 1–2 mL medium in a 24-well tissue culture plate.
2. Add 25 µL pre-washed and resuspended Macrobeads™ (*Table 1, below*).
3. Incubate in a humidified CO_2 incubator at 37°C, according to your specific experimental requirements.
4. Harvest the activated T cells and use directly for further analysis.
5. For flow cytometry analyses, remove the beads prior to staining. Place the tube on a magnet for 1–2 min to separate the beads from the solution. Transfer the supernatant containing the cells to a new tube.

EXPAND HUMAN T CELLS

1. Start with $1–1.5 \times 10^6$ purified T cells/mL in a culture medium in a suitable tissue culture plate or tissue culture flask.
2. Add Macrobeads™ at a bead-to-cell ratio of 1:1 (*see Table 1*).
3. Add 300 U/mL rIL-2. Store at 2-8˚C.
4. Incubate in a humidified CO_2 incubator at 37°C, according to experimental needs.
5. Examine cultures daily, noting cell size and shape. Cell shrinking and reduced proliferation are typically observed in exhausted cell cultures.
6. Count the cells at least twice weekly after thorough resuspension.
7. When the cell density exceeds 2.5×10^6 cells/mL or when the medium turns yellow, split cultures back to a density of $0.5–1 \times 10^6$ cells/mL in culture medium containing 300 U/mL rIL-2.

Products and Services
- Mouse Monoclonal Antibody
- Rat Monoclonal Antibody
- Human Antibody
- Hybridoma Sequencing
- Polyclonal Antibody

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RESTIMULATION

Cell cultures showing signs of exhaustion (typically at day 7–10 of expansion) can be restimulated several times by adding fresh Macrobeads™ and rIL-2. The CD8+ T cells remain cytotoxic after repeated restimulations. Restimulation is typically necessary when cell shrinking and a reduced rate of proliferation are observed.

Guidelines for restimulation are provided in Table 2. Optimize for your particular application. Do not use an excess volume of Macrobeads™.

1. Prior to restimulation, remove the used Macrobeads™ by transferring the cells to a suitable tube.
2. Place the tube in the magnet for 1–2 min.
3. Transfer the supernatant containing the cells to a new tube.
4. Split the cultures back to a density of 0.5–1 × 10^6 cells/mL in culture medium containing 300 U/mL rIL-2 and repeat the Expand procedure.

Table1. Volume recommendations for bead-to-cell ratio = 1:1

Type Of Culture Plate/Flask	24-Well Plate	175 cm^2 Tissue Culture Flask
Cell concentration	1X10^6 T Cells/Well	50X10^6 T Cells/Flask
Macrobeads™	25 µL	1,250 µL
rIL-2	300 U/mL	300 U/mL
Seeding volume (Medium)	1-2 mL	50-100mL

Table2. Restimulation guidelines for anti-CD3/CD28-expanded cultures

Specifications	1 X 10^6 T Cells
Cell type	Subsequent restimulations*
CD4+ (polyclonal)	8-11 day intervals
CD8+ (polyclonal)	7-10 day intervals
T cells	10-12 day intervals*

** Establish optimal times for your particular cells. Note that these are only generic guidelines.*

CD28/CD3 Cell Activation/Expansion Beads price		
2 mL	$499	PM-CAR2002-2ML
10 mL	$1,400	PM-CAR2002-10ML

Products and Services
- Mouse Monoclonal Antibody
- Rat Monoclonal Antibody
- Human Antibody
- Hybridoma Sequencing
- Polyclonal Antibody

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DATA

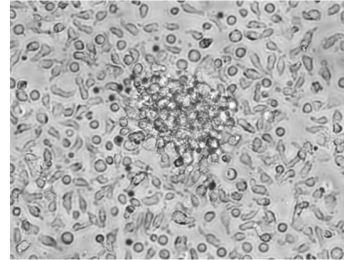

Figure 2. Morphology of Activated and expanded T cells stimulated with CD28/CD3 beads.

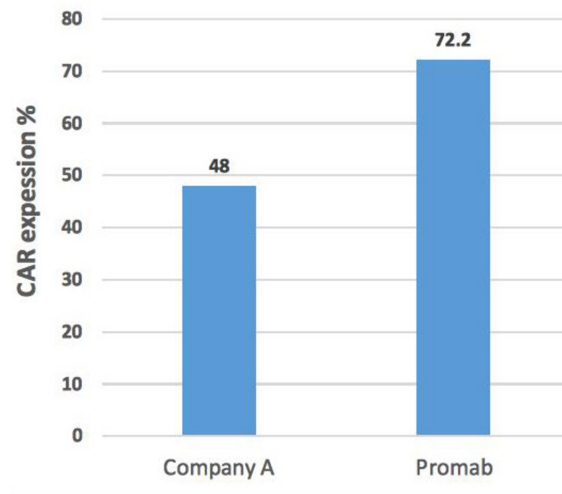

Figure 3. Transduction efficiency of CAR-T cells expanded with Company A and Promab's CD28/CD3 beads. CAR-T cells had >70% transduction efficiency when expanded with Promab's beads as detected by flow cytometry after staining cells with FAB antibody.

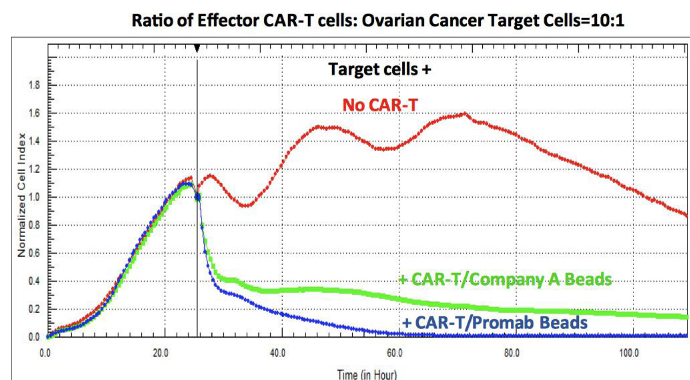

Figure 4. Real-time Cytotoxicity Assay (RTCA) with Promab and Company A beads. CAR-T cells expanded with Promab's beads had higher cytotoxic activity.