ProMab Biotechnologies, Inc.

Protocol

T CELL ACTIVATION AND EXPANSION

OVERVIEW

The purpose of this product is to activate and expand human T cells: CD4+ T cells,CD8+ T cells, antigen specific T cells or polyclonal T cells following CD3/CD28 Macrobeads™ (cat. # PM-CAR2002, ProMab Biotechnologies) activation using recombinant IL-2. The expanded T cells can be analyzed after activation (for transfection/transduction or for other biochemical assays: genomics, proteomics, and T cell functional assays). T cells can be cultured to differentiate into T helper cell subsets, or for T cell proliferation/expansion of Ag-specific or polyclonal T cells.

MATERIALS NEEDED

1. Buffer: Phosphate buffered saline with 0.1% bovine serum albumin and 2 mM EDTA, pH 7.4 (PBS w/0.1% BSA).
2. CD3/CD28 Macrobeads™ (cat. # PM-CAR2002, ProMab Biotechnologies).
3. Magnet
4. Culture medium:Advanced RPMI Medium 1640 with 2 mM L-Glutamine, 10% FCS/FBS and 100 U/ml penicillin/streptomycin can be used. Alternatively, Cancer Stem Premium™ (cat. # 20101, ProMab Biotechnologies) with 100 U/ml penicillin/streptomycin, or another equivalent culture medium.
5. Recombinant human IL-2 (cat. # Pr21269, ProMab Biotechnologies).
6. Heat inactivated Fetal Calf Serum (FCS).
7. Flat bottom tissue culture plates or tissue culture flasks.
8. Humidified CO_2 incubator.

WASHING OF MACROBEADS™ BEFORE USE

1. Resuspend the Macrobeads™ 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. Place the tube on a magnet for 1 min and discard the supernatant.
5. Remove the tube from the magnet and resuspend the washed Macrobeads™ in the same volume of culture medium as the initial volume of Macrobeads™ taken from the vial.

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ACTIVATION

1. Start with 8×10^4 purified T cells in 100–200 µL medium in a 96-well tissue culture plate.
2. Add 2 µL pre-washed and resuspended Macrobeads™ to obtain a bead-to-cell ratio of 1:1 (*see Table 1*).
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 applications, 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.

EXPANSION

1. Start with 1–1.5 × 106 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 30 U/mL rIL-2 (cat. # Pr21269, ProMab Biotechnologies). Store at 2-8˚C
4. Incubate in a humidified CO_2 incubator at 37°C, according to your specific 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 × 106 cells/mL or when the medium turns yellow, split cultures back to a density of 0.5–1 × 106 cells/mL in culture medium containing 300 U/mL rIL-2 (cat. # Pr21269, ProMab Biotechnologies).

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BEAD-TO-CELL RATIO

Type of culture plate/flask	24-Well Plate	175 cm^2 Tissue Culture Flask
Cell concentration	1×10^6 T cells/well	50×10^6 T cells/flask
MacroBeads™	25 µL	1,250 µL
rIL-2	300 U/mL	300 U/mL
Seeding volume (medium)	1–2 mL	50–100 mL

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