ProMab Biotechnologies, Inc.

Protocol

T CELL RESTIMULATION

OVERVIEW

Cell cultures showing signs of exhaustion (typically at day 7–10 of expansion) can be restimulated several times by adding fresh CD3/CD28 Macrobeads™ (cat. # PM-CAR2002, ProMab Biotechnologies) and recombinant IL-2 (cat. # Pr21269, ProMab Biotechnologies). The CD8+ T cells remain cytotoxic after repeated restimulations. Restimulation is typically necessary when cell shrinking, and a reduced rate of proliferation are observed.

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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RESTIMULATION

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 × 106 cells/mL in culture medium containing 300 U/mL rIL-2 and repeat the Expansion procedure. Guidelines for restimulation are provided in Table 1. Optimize for your application. Do not use an excess volume of Macrobeads™, as excess MacrobeadsTM may inhibit expansion.

RESTIMULATION GUIDELINES

Number of Cells	1×10^4 T-Cells
Cell type	Subsequent restimulations*
$CD4^+$ (polyclonal)	8-10 day intervals
$CD8^+$ (polyclonal)	7-9 day intervals
T cells	7-9 day intervals*

Table 1. Restimulation guidelines for anti-CD3/CD28-expanded cultures

T CELL ACTIVATION AND EXPANSION

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

Table 2. Volume recommendations for bead-to-cell ratio = 1:1