

Protocol

T CELL RESTIMULATION

OVERVIEW

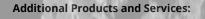
Cell cultures showing signs of exhaustion (typically at day 7–10 of expansion) can be restimulated several times by adding fresh CD3/CD28 Macrobeads[™] (cat. # PM-CAR2002, ProMab Biotechnologies) and recombinant IL-2 (cat. # Pr21269, ProMab Biotechnologies). The CD8+ T cells remain cytotoxic after repeated 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 CO₂ 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.
- 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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- Rabbit Monoclonal Antibody
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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





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T CELL ACTIVATION AND EXPANSION



BEAD-TO-CELL RATIO

| Type of culture plate/flask | 24-Well Plate | 175 cm² Tissue Culture Flask |
|--------------------------------|----------------------------------|---------------------------------|
| Cell concentration | 1 × 10 ⁶ T cells/well | 50 × 10⁰ 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

