BENAROYA RESEARCH INSTITUTE
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Tetramer Lab Core
Standard Protocols

Culturing T cell clones

1. Warm T cell medium (RPMI, 10% pooled human serum, 1% pen/strep) in a 37° C water bath.

2. Hold the cryovial containing the frozen T cell clones in the surface of the water bath with an occasional gentle "flick" during thawing, until only a small bit of ice remains in the cryovial.

3. Add warm T cell medium dropwise into the cryovial containing the cell suspension slowly. Transfer the diluted cell suspension to a 15 ml Falcon tube containing 10 mL of warm T cell medium for every vial of cells added.

4. Centrifuge the cells at 1200 rpm for five minutes.

5. Decant the supernatant. Resuspend the pellet in 10 mL of warm T cell medium and repeat step 4.

6. Resuspend the pellet in T cell medium. Determine the cell number with a Hemacytometer.

7. Resuspend the T cell clones at a concentration of 1 million per mL T cell medium. Plate 1 mL of T cell clone suspension in each well of a 48-well plate. Add 2.5 million irradiated feeder cells (don't have to be HLA-matched), IL-2 (10 U/mL final concentration), and PHA (2 µg/mL final concentration) to each well.

8. After 4-5 days of stimulation, split or maintain the T cell clones depending on the confluence. Add IL-2 to a final concentration of 10 U/mL.

9. Check the culture every other day. Split or maintain the culture accordingly.