Protocol: Ex Vivo Activation of Lymphocytes

Steps:

1. Sample collection and dispensing:
   a. Whole blood: dispense 50-200 μL per sample.
   b. Fresh PBMC: resuspend at 5-10 x 10^6 viable lymphocytes per mL in cRPMI-10, dispense 200 μL per sample.
   c. Cryopreserved PBMC: Follow PBMC Cryopreservation protocol, resuspend at 5-10 x 10^6 viable lymphocytes per mL in warm cRPMI-10, dispense 200 μL per sample. Rest at 37C for 6-18 h.

2. Stimulation:
   a. SEB: use at 1 μg/mL final concentration (see stock preparation table).
   b. Peptides: use at 1-2 μg/mL/peptide final concentration (see stock preparation table).
   c. Proteins or other complex antigens: titer and use at appropriate concentration.

3. Addition of secretion inhibitor:
   a. For most cytokines: use brefeldin A at 10 μg/mL final concentration (see stock preparation table).
   b. For CD107 and CD154: use monensin at 10 μg/mL final concentration (see stock preparation table).
   c. For assays combining cytokines and CD107 or CD154: use brefeldin A and monensin at 5 μg/mL final concentration each.

4. Addition of labeled Ab (for CD107 and CD154 ONLY):
   a. Add recommended titer of labeled antibody and protect from light.

5. Addition of costimulatory antibodies (optional):
   a. Add 1 μg/mL final concentration of CD28 and/or CD49d (labeled antibody can be used if analysis of the marker is desired).

6. Incubation:
   a. For CD107 and CD154: 4-6 h at 37C.
   b. For IL-10 and TGFβ: 12-24 h at 37C.
   c. For all other cytokines: 6-12 h at 37C.

Notes:

1. Activate in either 15-mL conical polypropylene tubes (laid on 5° slant for PBMC), or 96-well conical polypropylene plates.

2. Results from PBMC may be slightly superior to whole blood, but cryopreservation and/or shipping reduces responses, particularly for whole protein antigens.

3. Crypreserved PBMC should have a redovery of >60% and viability >80% for consistent results.

4. Cryopreserved PBMC can also be rested in bulk, then recounted and dispensed.

5. Costimulatory antibodies can add to background cytokine production, but generally increase responses (especially for whole protein antigens, but also for peptides).

6. Use non-FCS-containing media (e.g., AIM-V) for TGFβ assays.

7. Incubation can be automated using a programmable incubator or water bath that cools samples to 4-18C at the end of the desired activation period (use 18C for whole blood to avoid platelet aggregation). CO_2 supplementation is not necessary if HEPES-buffered medium is used.

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Stock Solution Recommendations:

Solution	Stock Concentration	Intermediate Dilution	Final Concentration
Brefeldin A[1]	5 mg/mL in DMSO (store in aliquots at -20C)	1:10 in PBS	10 μg/mL (1:50) or 5 μg/mL (1:100) when combined with monensin
Monensin[1]	5 mg/mL in ethanol (store at -20C)	1:10 in PBS	10 μg/mL (1:50) or 5 μg/mL (1:100) when combined with brefeldin A
Peptide mixes[1]	0.5-1 mg/mL/peptide in DMSO (store in aliquots at -20C)	1:10 in PBS	1 μg/mL/peptide (1:50 - 1:100)
SEB	50 μg/mL in PBS	None	1 μg/mL (1:50)

[1]It is important to avoid solvent toxicity. Final DMSO+ethanol concentration from all sources (peptides, brefeldin A, monensin) should not exceed 0.5%.