MSC Analysis

MSC Immune Modulation on T cells

1. Thaw and seed MSCs at 3,000 cells/cm^2 in six-well plates. ***See Thawing Procedure on Page 8***.
2. Allow MSCs to reach 80% confluence before adding the peripheral blood mononuclear cells (PBMCs) (approximately 2–3 days). MSCs should be approximately 500,000 cells per well before proceeding.
3. Pre-warm complete RPMI Medium 1640 (Irvine Scientific, Catalog # 9161) containing 10% fetal bovine serum (MSC qualified) to 37°C for no more than 30 minutes.
4. Thaw PBMCs according to vendor's instructions. Incubate cells at 37°C, 5% CO_2 for 3 hours to recover.
5. After recovery, stain PBMCs with CellTrace™ CFSE according to manufacturer's instructions.
6. After staining, wash the PBMCs three times using fresh, room temperature complete RMPI medium.
7. On the last wash, resuspend the PBMC cell pellet in minimal volume of complete RMPI medium.
8. Based on MSC cell counts, co-culture labeled PBMCs with MSCs in a six-well plate in a 1:10 ratio (MSC:PBMCs).
9. Add phytohemagglutinin to a final concentration of 20µg/mL in a total volume of 4mL complete RPMI per well.
10. Prepare a negative control sample by plating 250,000 PBMCs per well in a six-well plate with 4mL complete RPMI medium.
11. Prepare a positive control sample by plating 250,000 PBMCs per well in a six-well plate with 4mL complete RPMI medium supplemented with a final concentration of 20µg/mL phytohemagglutinin.
12. On day three and day five of co-culture, PBMC derived CD3$^+$ cell proliferation can be measured by using a flow cytometer with 488nm excitation and emission filters appropriate for fluorescein.