**ImmunoCult™ Human Treg Differentiation Supplement**

**Serum-free culture supplement for the differentiation of human naïve CD4+ T cells into regulatory T cells (Tregs)**

**Catalog # 10977**
**1 mL**

**Product Description**

ImmunoCult™ Human Treg Differentiation Supplement contains a human cytokine and small molecule formulated to promote the robust activation, expansion, and differentiation of peripheral blood-derived, naïve, CD4+ human T cells into regulatory T cells (Tregs).

ImmunoCult™ Human Treg Differentiation Supplement is intended for use with ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981) and ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971).

- Formulated for the induction of human Treg differentiation
- Optimized for use with ImmunoCult™-XF T Cell Expansion Medium
- Supports higher levels of memory T cells in comparison to competitor Treg differentiation kit
- Supports up to 20% higher proportions of CD4+CD25+FOXP3+ T cells in comparison to competitor Treg differentiation kit
- Supplied as a 50X concentrate; after thawing and mixing, the tube contents can be added directly to medium

**Properties**

- **Storage:** Store at -20°C.
- **Shelf Life:** Stable for 12 months from date of manufacture (MFG) on label.
- **Contains:**
  - Recombinant human TGF-β1
  - All-trans retinoic acid

**Handling / Directions For Use**

**PREPARATION OF COMPLETE Treg DIFFERENTIATION MEDIUM**

Use sterile techniques to prepare complete Treg Differentiation Medium (ImmunoCult™ Human Treg Differentiation Supplement + ImmunoCult™-XF T Cell Expansion Medium). The following example is for preparing 50 mL of complete medium. If preparing other volumes, adjust accordingly.

1. Thaw ImmunoCult™ Human Treg Differentiation Supplement at room temperature (15 - 25°C) until just thawed. Mix thoroughly.
   
   **NOTE:** If necessary, centrifuge for 30 seconds to recover liquid from cap.
   
   **NOTE:** If not used immediately, store supplement at 2 - 8°C for up to 1 month. Do not re-freeze.

2. Add 1 mL of ImmunoCult™ Human Treg Differentiation Supplement to 49 mL of ImmunoCult™-XF T Cell Expansion Medium (Catalog #10981). Mix thoroughly.

**DIFFERENTIATION TO Tregs**

1. Start with purified human naïve CD4+ T cells at 1 x 10^6 cells/mL in complete Treg Differentiation Medium.
2. Activate human naïve CD4+ T cells using ImmunoCult™ Human CD3/CD28 T Cell Activator (Catalog #10971) or other desired reagents to activate T cells. Incubate cells at 37°C and 5% CO₂.
3. Adjust the cell density to 1 x 10^6 cells/mL every 3 - 4 days as needed with the addition of fresh complete Treg Differentiation Medium for 7 days.
4. On Day 7, differentiated Tregs are ready to be used in the desired application.