

Protocol for the Cell Stimulation Cocktail

The Cell Stimulation and Protein Transport Inhibitor Cocktail is a pre-mixed cocktail used to induce cytokine production and inhibit their intracellular transport for flow cytometry detection. The solution is composed of an optimized mixture of brefeldin A, ionomycin, monensin, and PMA. PMA and ionomycin induce cytokine production in many cell types and brefeldin A and monensin are protein transport inhibitors.

1. Resuspend the cells of interest in cell culture medium at $1-10 \times 10^6$ cells /mL.
2. Add 2 uL of the cocktail per each mL of suspended cells to reach a 1X concentration.
3. Incubate cells for 6-16 hours in a CO₂ incubator.
4. Activated cells may be used for intracellular and surface flow cytometry analysis.