Biological Activity Assay for Gibco™ Recombinant Proteins – Cytokine Production Assay

Cytokines produced by cultured cells are generally quantified by ELISA method. This method describes dose-dependent co-stimulation of secretion of IFN-γ by human natural killer cells responding to human IL-12 in the presence of human IL-18 (this method is based on Kannan, Y et al, 2011, Blood 117:2855–2863).

Method

1. Human PBMC isolation is described in detail in the chemotaxis assay.
2. CD56+ human NK cells were isolated from PBMC by magnetic beads method as described by the manufacture (Mitenyi Biotech).
3. Human NK cells were plated into a 96-well plate at 2 x 10^6/mL (200 µL/well) in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin, 10 ng/mL of human IL-18 with different concentration of human IL-12 (0–20 ng/mL).
4. Incubate cells in 37°C incubator with 5% CO2 for 24–48 hours.
5. Collect cell culture supernatant (50 µL/sample) for ELISA assay.
6. Carry out ELISA assay for human IFN-γ as described by the manufacture (eBioscience BMS228).