The Activation of Human Neutrophils

Activation of neutrophils is not an all or nothing phenomenon and each function has its own threshold for a response. Please choose the proper stimulus and proper concentration of the stimulus for your experiment design.

Materials Needed

- Culture Medium:
  - RPMI-1640 supplemented with 1-10% autologous serum
- Washing buffer:
  - PBS without calcium or magnesium
  - 0.5% Human Serum Albumin (HSA)
  - 2mM EDTA
- Examples of cytokines that can be used for stimulus:
  - Interleukin 8 (IL-8)
  - Leukotriene B4 (LTB4)
  - Platelet-activating factor (PAF)
  - C5a
  - Interleukin 1 (IL-1)
  - Tumor necrosis factor alpha (TNF-α)
  - Kallikrein
  - Formylated peptide fMLP
  - Phorbol myristate acetate (PMA)
  - PMA with ionomycin
- PBS without calcium or magnesium
- PBS with calcium and magnesium
- Trypan Blue
- Hemocytometer
- Microscope
- Cell culture flask or 24-well, 48-well, 96-well cell culture plate

Protocol

1. Thaw cryopreserved neutrophils according to the HemaCare “How to Thaw Cryopreserved Cells” protocol.
2. Re-suspend the post-thaw neutrophils at 1-5 × 10^6 cells/ml.
3. Evaluate the cell viability and cell yield of the post-thaw neutrophil by 0.4% trypan blue exclusion method, using a hemocytometer and a microscope. Evaluate cell purity of the post-thaw neutrophil by flow cytometer.
4. Plate cells at desired concentration. Add stimulus of choice into neutrophil culture as the experimental group. For example PMA (5ng/ml) or PMA (5ng/ml) + ionomycin (250ng/ml) as the experimental group and PBS with calcium and magnesium as the experimental control group.

Protocol

5. Put the culture system at 37°C in a humid atmosphere with 5% CO₂ for 15 min. Please note: incubation time will need to be adjusted according to the chosen stimulus and experiment design.
6. Assess the activation (calcium mobilization, migration, oxidative burst, phagocytosis, or degranulation) of neutrophil according to experiment design.

Notes:

- Always wear personal protective equipment and use universal precaution when working with human-derived biological materials and liquid nitrogen.

Disclaimer:

Neutrophils are a very sensitive cell type and can be easily activated by reagents. HemaCare does not send cells that have been activated.