# 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.

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

## 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
- ☐ PBS with calcium and magnesium
- ☐ Trypan Blue
- ☐ Hemocytometer
- ☐ Microscope
- ☐ Cell culture flask or 24-well, 48-well, 96-well cell culture plate
- ☐ 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
  - ☐ Formulated peptide fMLP
  - ☐ Phorbol myristate acetate (PMA)
  - ☐ PMA with ionomycin

## Notes

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

## Protocol

1. Thaw cryopreserved neutrophils according to the Charles River *"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.*
5. Put the culture system at 37°C in a humid atmosphere with 5% $CO_2$ 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.

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