




## RESPIRATORY BURST ACTIVITY



### REAGENT PREPARATION





#### Assay Buffer:

-  500 ml RPMI 1640 Cell Culture Medium
-  5 g Bovine Serum Albumin (BSA)
-  500 µl 1M Calcium Chloride

#### PMA:

-  200 nM PMA in DMSO


#### Red Blood Cell Lysis Buffer:

-  9 ml ddH<sub>2</sub>O
-  0.734 g NH<sub>4</sub>Cl
-  0.077 g NaHCO<sub>3</sub>
-  0.039 g EDTA




### SAMPLE PREPARATION

#### Blood:

-  Freshly collected with anticoagulants.

#### Cells:

-  Suspend the cells in Assay buffer to a concentration of 10<sup>6</sup> cells/ml. Keep the cells at 37°C for at least 15 minutes.



### ASSAY PROTOCOL

- 1 Add 10 µl of sample to a tube
- 2 Add 10 µl of DHR123 to the tube
- 3 15 min Incubate at 37°C
- 4 Add 25 µl of PMA/PBS/other
- 5 45 min Incubate at 37°C
- 6 Add 2 ml of Red blood cell lysis buffer
- 7 3-20 min Incubate at 37°C
- 8 10 min Centrifuge at 500 xg at RT
- 9 Remove supernatant
- 10 Resuspend cell pellet in 0.5 ml assay buffer
- 11 Measure by flow cytometry with a fluorescence emission at 530 nm

