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Orion II Microplate Luminometer Simplicity V4.20

ABEL® Cell Activation Assay for Whole Blood or Isolated Cells with Pholasin® and Adjuvant-K™

The ABEL® (Analysis By Emitted Light) Cell Activation Assay is a chemiluminescent test that measures degranulation and the real-time production of free radicals during the activation of the NADPH oxidase system, the so-called 'respiratory burst'. The NADPH oxidase is most developed in granulocytic leucocytes, in particular neutrophils and monocytes, in which electrons are transported through the cell membrane to reduce oxygen to the free radical, superoxide anion O^{2-}. Superoxide is released to the outside of the cell on initial activation and, during phagocytosis, into a phagosomal vacuole. The assay uses both the light-emitting protein Pholasin®, which reacts with free radicals to emit light, and an enhancer, Adjuvant-K™.

The assay can be used to:

- ❑ measure the respiratory burst in leucocytes
- ❑ study abnormalities in NADPH oxidase such as occur in different types of chronic granulomatous disease and various polymorphisms
- ❑ distinguish between receptor activation and intracellular activation of NADPH oxidase
- ❑ monitor degranulation of myeloperoxidase
- ❑ measure free radicals and oxidants produced by a range of cell types including brain cells
- ❑ monitor changes in the activation of the NADPH oxidase in response to complement activation, infection, inflammation and medical intervention
- ❑ the assay thus has applications in:
  - biocompatibility monitoring
  - drug evaluation and testing
  - QC of vaccines
  - surgery
  - disease management
  - the assessment of neutrophil activity following, for example, chemotherapy or transplantation.

The test is simple, rapid and ultra-sensitive and capable of measuring fmols of superoxide from a small number of cells. The assay works on venous or capillary blood as well as on isolated cells and has been used on blood from humans, horses, cattle, dogs, cats, rats, mice, birds, fish and reptiles. The amount of blood required for a 1mL assay is 1µL but it is recommended to do an initial 1:100 dilution of 20µL.

The assay is available as a test kit that includes all the reagents, tubes in which to dilute the blood and two stimulants to initiate the respiratory burst: the receptor stimulant fMLP (f-Met-Leu-Phe), which activates NADPH oxidase via receptors on the surface, and the phorbol ester PMA that enters the cell and activates NADPH oxidase via the activation of protein kinase C. Other activators or receptor primers such as platelet activating factor or lipopolysaccharide can be used in place of, or together with, fMLP. Many non-human leucocytes do not have receptors for fMLP. In the presence of Pholasin® and Adjuvant-K™ the kinetics of this response can be determined by measurement of the light emitted during the few minutes of the analysis

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Materials:

Luminometer: Orion II Microplate Luminometer
Software: Simplicity V4.20
Assay: ABEL® Cell Activation Test Kit with Pholasin® and Adjuvant-K™, Knight Scientific Ltd, Plymouth, UK (Product No. KSL-ABEL-04M)

Method:

For more detailed protocols please refer to the kit instructions supplied by the manufacturer. A product support document for the ABEL® Cell Activation assay can be downloaded from http://www.knightscientific.com/downloads.

1. Reconstitute the reagents according to the kit instructions.
2. Prime the automatic reagent injectors that must be capable of operating in the measurement position (each injector has to be primed with at least 4 x 150µL of the respective solution).
    a. prime injector 2 with fMLP
    b. prime injector 4 with PMA
3. Dilute whole blood to 1:100 in 2 mL by adding 20µL of anti-coagulated blood (preferably EDTA) to 2mL of blood dilution buffer. For controls, plasma is used in place of blood.
4. To each well add 20 µL diluted blood (or diluted plasma control), 90 µL assay buffer, 50 µL reconstituted Pholasin® (0.5 µg), 20 µL Adjuvant-K™.
5. Incubate the microplate in the luminometer at 37°C for 6 minutes.
6. Create a master protocol in the Simplicity software (Fig.1.).

First create raw data protocols as follows:

a) 1 second of orbital shaking (maximum = 1440min^{-1}) + 0.2 second measurement
b) 0.2 second measurement
c) 20 µL injection (using in Meas-position)

Using the batch protocol build these functions into a master protocol. For optimum results and minimum cycle time we recommend running no more than 6 wells simultaneously. The number of repeats may need adjusting depending on the number of wells used. In brief the protocol consists of:

-shaking of the plate
-light measured for approximately 2.5 minutes
-20µL of stimulant injected into each well
-light measured for approximately 5 minutes (if using fMLP) or 7 minutes (if using PMA)

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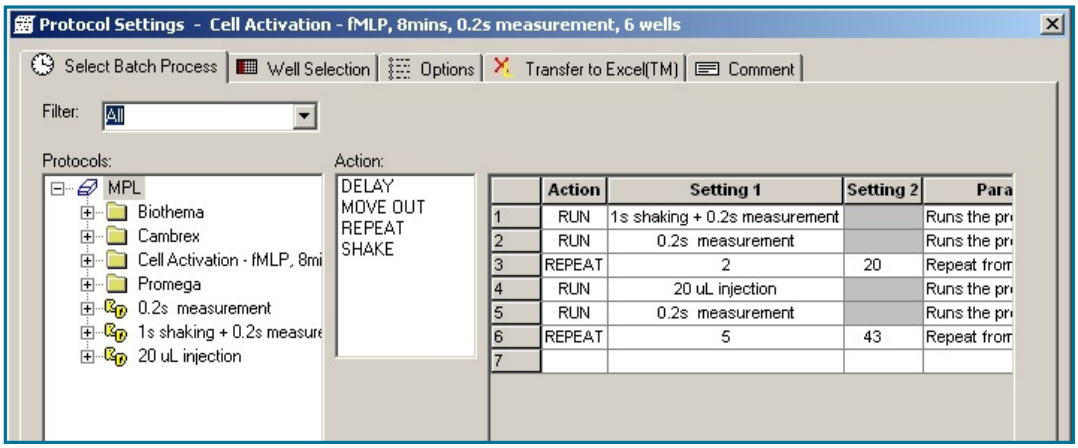

Figure 1: Batch Protocol settings in Simplicity 4 software when using fMLP as a stimulant and running 6 wells simultaneously

Example:

Leucocyte activation was measured in whole blood from two healthy adult donors. Capillary blood samples were taken by finger prick using a safety lancet (Sarstedt) and stored in Microvette® tubes containing EDTA anticoagulant (Sarstedt). Blood plasma was used as a negative (no cell) control. The assay was performed as described above with samples assayed in duplicate to show the reproducibility of the assay.

Results:

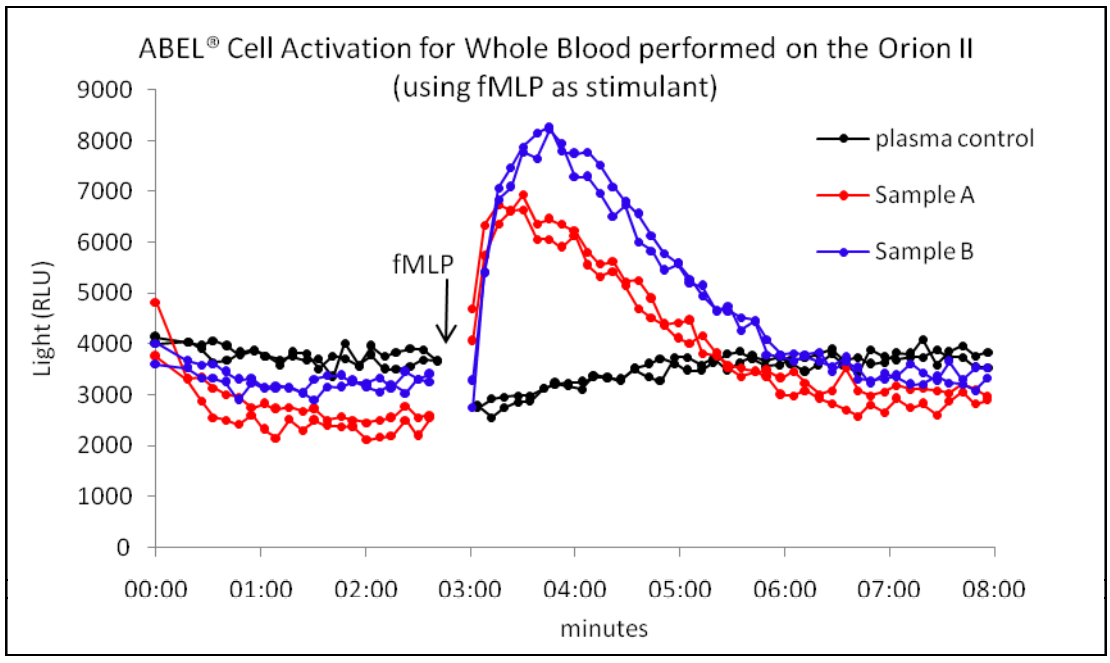

Figure 2. Free radical production by activated leucocytes in whole blood samples from 2 individuals monitored by the luminescence of Pholasin®. The cells are activated by the injection of the stimulant fMLP after 2minutes 50 seconds. The black line represents the plasma (no cell) control.

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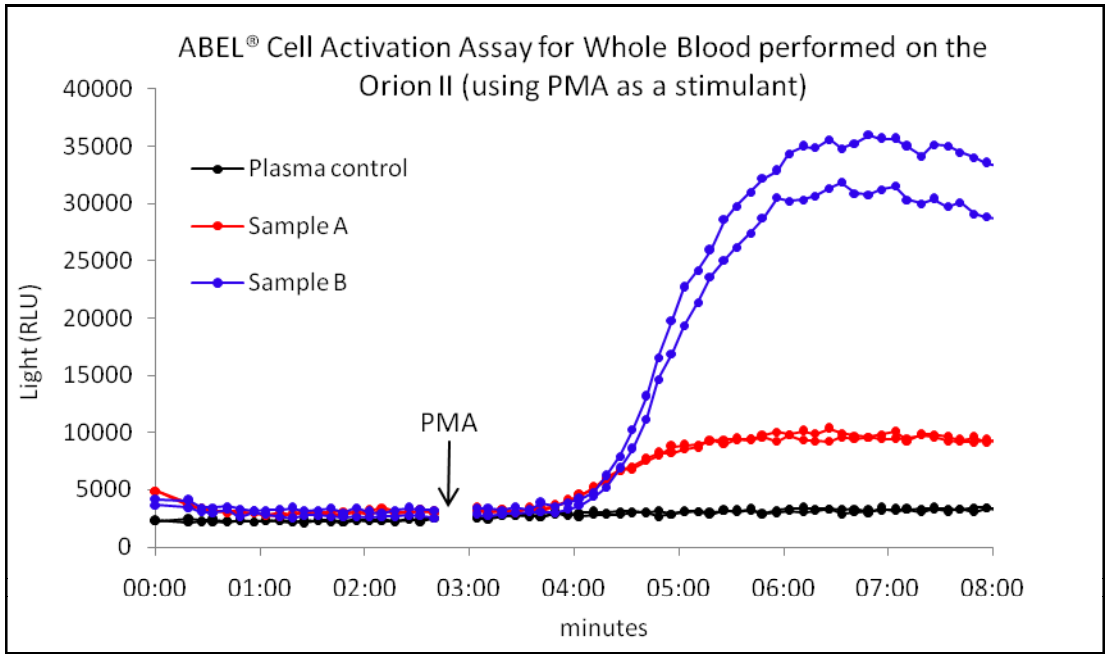

Figure 3. Free radical production by activated leucocytes in whole blood samples from 2 individuals monitored by the luminescence of Pholasin®. The cells are activated by the injection of the stimulant PMA after 2 minutes 50 seconds. The black line represents the plasma (no cell) control

Summary:
The activation of leukocytes present in the blood can be demonstrated through the luminescence produced by Pholasin® and Adjuvant-K™ in response to free radical production. The kinetics of the NADPH oxidase system can be monitored and can identify if cells have been activated and/or primed and give a preview of the way cells, circulating in the peripheral blood, are likely to respond at a site of inflammation.

The Orion II microplate luminometer showed an excellent ability to detect the production of reactive oxygen species produced by living cells from a small sample (20 µL) of capillary blood.

Acknowledgement:

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Figures and text by courtesy of Knight Scientific Ltd., UK

Knight Scientific Ltd

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