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# Orion II Microplate Luminometer Simplicity 4

## Abel® Antioxidant Assay with Superoxide

This assay is a chemiluminescent test for measuring the capacity of a sample of fluid such as water, plasma, serum, synovial fluid, etc. to scavenge free radicals such as the superoxide anion.
Superoxide is generated in the assay instantaneously when solution B is injected into a microplate well containing solution A. If Pholasin® is present when the superoxide is generated, light will be emitted.
If there are antioxidants in the sample capable of scavenging superoxide then these will compete the Pholasin® for the superoxide and less light will be detected.

## Materials

| | |
|---|---|
| Luminometer: | Orion II Microplate Luminometer, equipped with 3 injectors |
| Software: | Simplicity 4 |
| Assay : | Abel® Antioxidant Assay with Superoxide, Knight Scientific Ltd., UK |
| Microplates: | Opaque microplates (solid, white, 96 well), supplied by Greiner |

## Method

Reconstitute all reagents as described in the kit insert, for detailed assay instructions please refer to:
http://www.knightscientific.com/pdfdir/ABEL-21M2.pdf

1. Preparing automatic reagent injectors:
Each injector has to be primed with at least 3 x150µl of the respective solution

a. Connect the reconstituted Pholasin® to injector 1 and prime
b. Connect Solution B to injector 2 and prime
c. Connect solution A to injector 3 and prime

2. Create the protocol in Simplicity 4 Software
Select a Fast Kinetic protocol and set the following parameters:

Inject 50 µl Pholasin with Injector 1
Inject 100 µl of solution A with injector 3
After a 10 second delay inject 25 µl of solution B

Read the light emission for 30 seconds.

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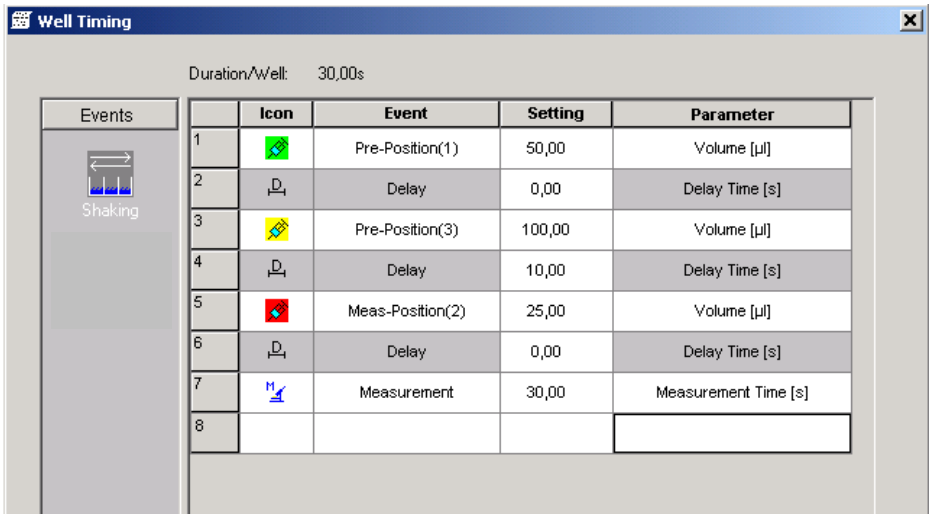


| | Icon | Event | Setting | Parameter |
|---|---|---|---|---|
| 1 | | Pre-Position(1) | 50,00 | Volume [µl] |
| 2 | | Delay | 0,00 | Delay Time [s] |
| 3 | | Pre-Position(3) | 100,00 | Volume [µl] |
| 4 | | Delay | 10,00 | Delay Time [s] |
| 5 | | Meas-Position(2) | 25,00 | Volume [µl] |
| 6 | | Delay | 0,00 | Delay Time [s] |
| 7 | | Measurement | 30,00 | Measurement Time [s] |
| 8 | | | | |

Figure 1: Parameter Settings in Simplicity 4 Software

## Example:

Nutritional supplements (named as Product R), with 3 different known concentrations have been measured.
10µl of each sample + 15µl assay buffer were pipetted into the wells of the microplate.
The protocol was processed as described above.

## Result:

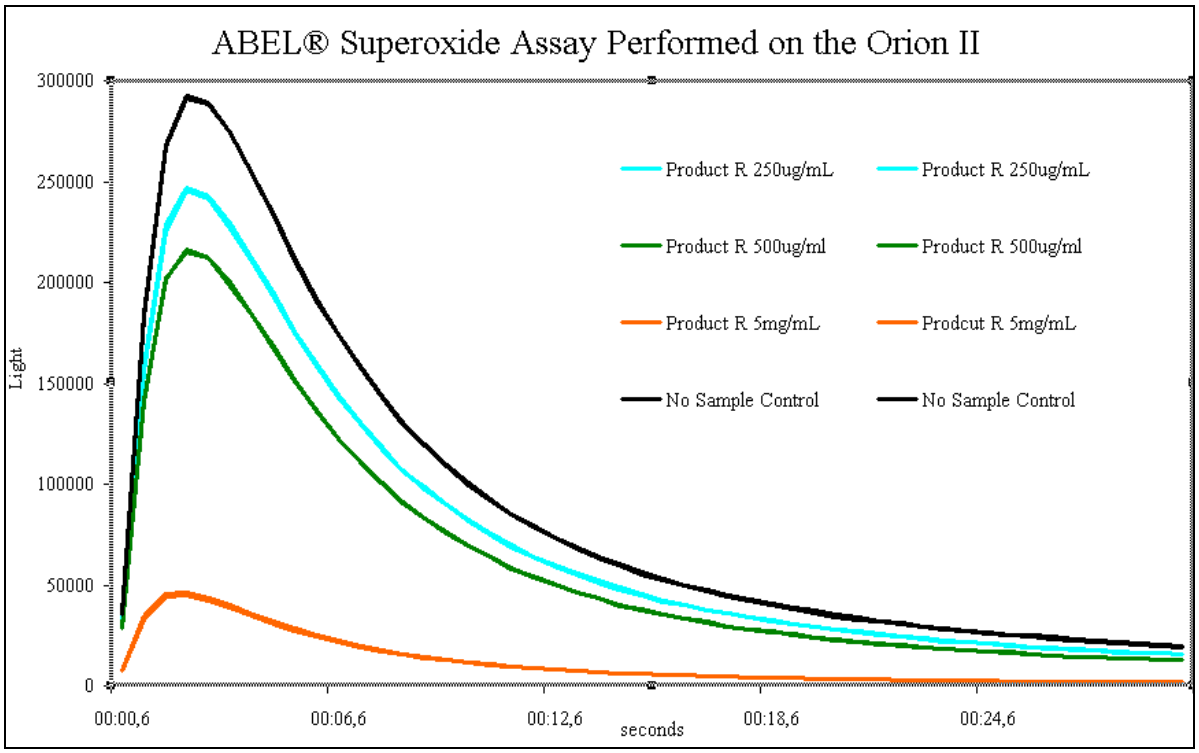


Figure 2: Light emission over 30 seconds for no sample control and three different sample concentrations.

Application Note 2006/03

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**Summary:**
The antioxidant capacity of the samples can be expressed as percentage inhibition of the no sample control or preferably as an *ABEL_RAC* mg Score (*Analysis By Emitted Light Relative Antioxidant capacity*). This score is calculated by the formula 1/EC50 x 100. The EC 50 is the amount of sample required (mg) to reduce the peak of light by half.

The sensitivity of the Orion II is excellent at both high and low ranges.

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

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