

Berthold Detection Systems GmbH  
Bleichstrasse 56–68  
D-75173 Pforzheim/Germany

Phone: +49(0)7231/9206-0  
Fax: +49(0)7231/9206-50  
E-Mail: [contact@berthold-ds.com](mailto:contact@berthold-ds.com)  
Internet: [www.berthold-ds.com](http://www.berthold-ds.com)

## Orion II Microplate Luminometer Simplicity 4

### Comparison of standard curves for ATP

Preparing standard curves is the best way to show detection limits and linearity of analytical techniques. In bio- and chemiluminescence analysis these parameters are influenced both by the instrument and the composition of the reagents. This application note compares standard curves prepared with ATP Reagent SL (stable light) and ATP Reagent HS (high sensitive) performed on Orion II with respect to their linearity and detection limit.

### Reaction



The oxidation of luciferin by luciferase is ATP dependent and produces light, that can be measured in a luminometer. The light produced is proportional to the ATP concentration of the sample when ATP is the limiting factor of the reaction.

### Materials

**Luminometer:** Orion II Plate Luminometer  
**Software:** Simplicity 4  
**Microplates:** opaque microplates (solid, white, 96 well)  
**Reagents:** ATP standard (Bio Thema 48-051)  
ATP diluent (Bio Thema 40-500)  
ATP reagent SL (Bio Thema 11-501)  
ATP reagent HS (Bio Thema 12-101)

Luminometer and software are from Berthold Detection Systems GmbH. The assay components are supplied by Bio Thema, the microplates by Corning.

### Methods:

Reagents were reconstituted as recommended by the manufacturer. The ATP standards were prepared by 10 fold dilutions of ATP standard with ATP diluent. The assays were performed at ambient temperature (22°C).

### Comparison of standard curves for ATP

#### For ATP SL reagent:

The injection lines were prepared by priming an injector in preposition with ATP SL reagent.

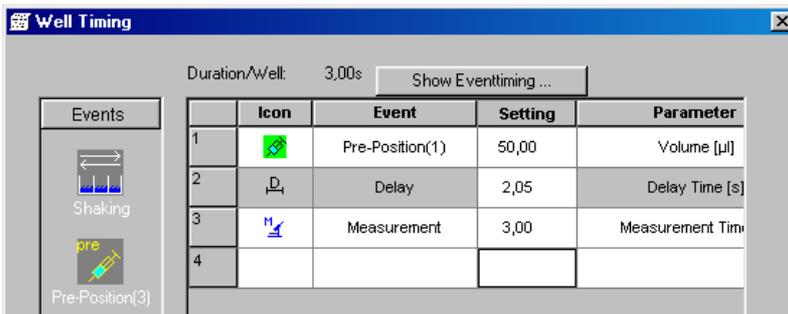
Standards were diluted from an ATP standard to concentrations of  $10^{-6} - 10^{-11}$  mol/l. 50µl aliquots of standards were put in the wells as triplicates. The chemical background was measured in multiple replicates. Chemical background and lowest dilutions were performed first. The automatic reagent injector was programmed to inject 50µl of SL reagent.

Delay time after injection: 2.05s

Measurement time: 3s

#### PC settings for ATP determination with SL reagent:

- ◆ Create a Raw Data protocol
- ◆ Select microplate format for 96 wells
- ◆ Define well timing



- ◆ Select wells you want to measure.
- ◆ Select to measure the background.
- ◆ Select if you want an automatic Excel transfer and save the protocol.

#### For ATP HS reagent

The injection lines were prepared by priming an injector in preposition with ATP HS reagent.

Standards were diluted from an ATP standard to concentrations of  $10^{-6} - 10^{-11}$  mol/l. 25µl aliquots of standards were put in the wells as triplicates. The chemical background was measured in multiple replicates. Chemical background and lowest dilutions were performed first. The automatic reagent injector was programmed to inject 100µl of HS reagent.

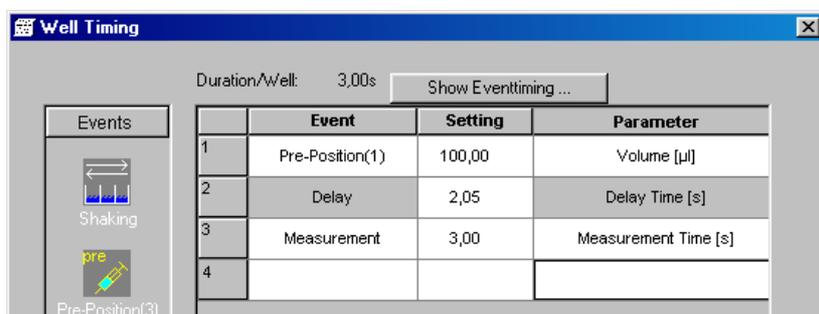
Delay time after injection: 2.05s

Measurement time: 3s

#### PC settings for ATP determination with HS reagent

- ◆ Create a Raw Data protocol
- ◆ Select microplate format for 96 wells
- ◆ Define well timing
- ◆ Select wells you want to measure.
- ◆ Select to measure the background.
- ◆ Select if you want an automatic Excel transfer and save the protocol.

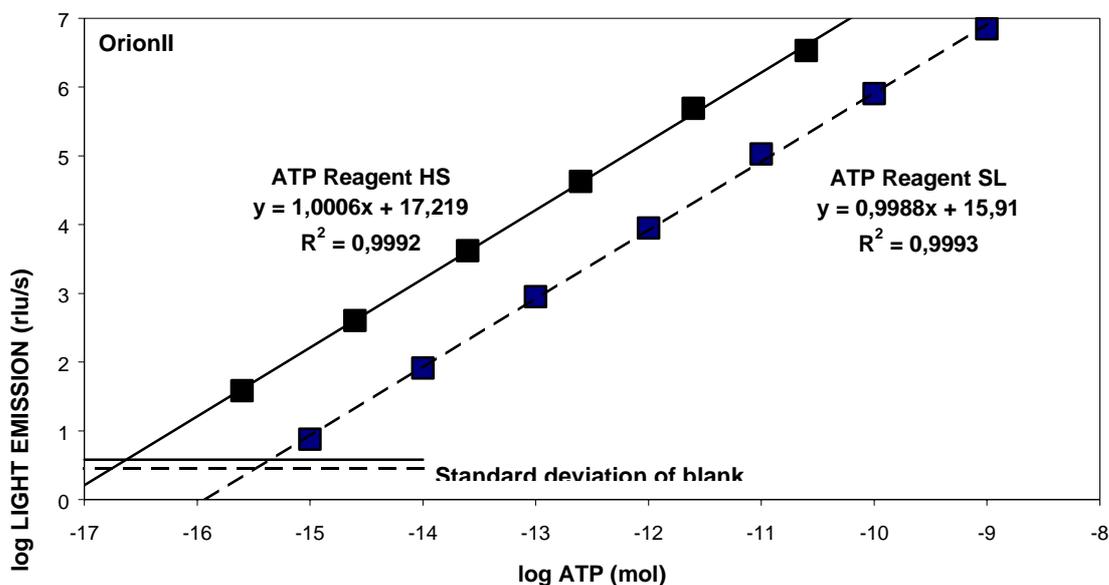
### Comparison of standard curves for ATP



Event	Setting	Parameter
1 Pre-Position(1)	100,00	Volume [µl]
2 Delay	2,05	Delay Time [s]
3 Measurement	3,00	Measurement Time [s]
4		

### Results

The relative light units per second (RLU/s) after subtraction of background value were plotted against the concentration of the standards in mol/tube. The double logarithmic plot below shows the standard curves for ATP with ATP Reagent SL and ATP reagent HS.



**Detection Limit for ATP with**  
**ATP Reagent SL: 402 amol/well**  
**ATP Reagent HS: 68 amol/well**

Both assays gave a linear response over a wide range of decades. The choice between ATP Reagent SL and ATP Reagent HS depends on what is preferred, stable light or high sensitivity.