

SpectraMax Microplate Readers

Comparing luminescence sensitivity of SpectraMax microplate readers using a homogeneous ATP assay

Assay used: ATPlite 1step Luminescence Assay System (PerkinElmer cat. #6016736)

ATP standards ranging from 1 μ M to 1 pM were prepared as a 10-fold serial dilution series in phosphate-buffered saline (PBS). 50 μ L of standard was pipetted into the wells of a white 96-well half-area microplate (Corning cat. #3693; n = 4 for each standard). Blank wells containing 50 μ L culture medium only (n = 8) were also prepared. 50 μ L of ATPlite 1step reagent was added to each standard and blank well. Contents of wells were mixed for 2 minutes using a plate shaker. Luminescence was then measured using the microplate readers listed. An integration time of 1 second was used for each reader. Standard curves were plotted using SoftMax[®] Pro Software (Figure 1).

To assess the sensitivity of each microplate reader, a plate was set up with 32 wells containing PBS only (blank) and four wells containing 50 nM ATP (sample). The lower limit of detection (LLD) for ATP was estimated for each microplate reader using the following formula: $LLD = 3 \times SD_{blank} \times \text{Concentration} / (RLU_{sample} - RLU_{blank})$.

Results are shown in Table 1.

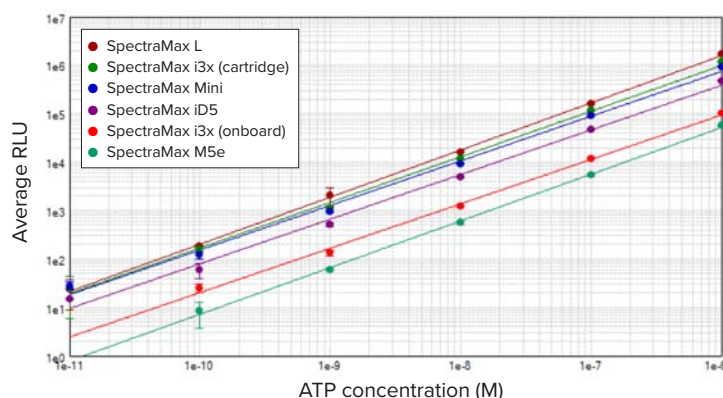


Figure 1. ATP standard curves read on SpectraMax microplate readers.

Instruments compared

- SpectraMax i3x
 - Onboard luminescence
 - Detection cartridge (LUM 96)
- SpectraMax iD5
- SpectraMax L
- SpectraMax M5e
- SpectraMax Mini

Instrument	ATP LLD (pM)	ATP LLD (moles)
SpectraMax i3x, luminescence detection cartridge LUM96	59.2	5.9×10^{-15}
SpectraMax i3x, onboard luminescence	121.3	1.2×10^{-14}
SpectraMax iD5	81.3	8.1×10^{-15}
SpectraMax L	36.5	3.6×10^{-15}
SpectraMax M5e	118.1	1.2×10^{-14}
SpectraMax Mini	17.8	1.8×10^{-15}

Table 1. Lower limits of detection for ATP determined using the ATPlite 1step assay with SpectraMax microplate readers.

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