

SpectraMax MICROPLATE READERS



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

Instruments compared:

- SpectraMax® iD5
- SpectraMax® i3x
- SpectraMax® M5
- SpectraMax® L

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

ATP standards ranging from 1 μM to 1 pM were prepared as a 10-fold serial dilution series in phosphate-buffered saline (PBS). 50 μL 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 μL culture medium only ($n = 8$) were also prepared. 50 μL 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 above. 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:

$$\text{LLD} = 3 * \text{SD}_{\text{blank}} * \text{Concentration} / (\text{RLU}_{\text{sample}} - \text{RLU}_{\text{blank}}).$$

Results are shown in Table 1.

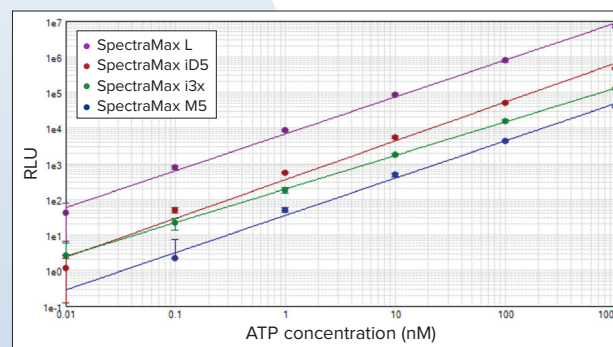


Figure 1. ATP standard curves read on SpectraMax microplate readers.

Instrument	ATP LLD (pM)	ATP LLD (moles)
SpectraMax L	26.8	2.7×10^{-15}
SpectraMax iD5	12.2	1.2×10^{-15}
SpectraMax i3x (onboard luminescence)	29.4	2.9×10^{-15}
SpectraMax i3x (luminescence detection cartridge, 96 wells)	11.9	1.2×10^{-15}
SpectraMax M5	173.8	17.4×10^{-15}

Table 1. Lower limits of detection for ATP using the ATPlite 1step assay, calculated for several SpectraMax microplate readers.

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