Evaluation of Transcreener® ADP² FI Assay on the Hidex Sense Platereader

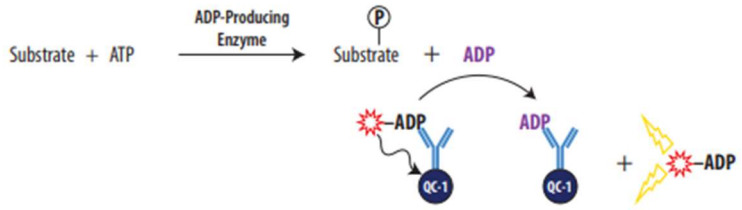
Assay principle

The Transcreener® assay platform is designed specifically for high-throughput screening (HTS), with a single-addition, mix-and-read format. It offers reagent stability and compatibility with commonly used multimode plate readers. The generic nature of the Transcreener® HTS assay platform eliminates delays involved in assay development for new HTS targets and greatly simplifies compound and inhibitor profiling across multiple target families.

The Transcreener® ADP² FI Assay extends the Transcreener® platform for ADP detection by utilizing a simple fluorescent intensity (FI) output. It can be used on fluorescence readers typically found in academic and therapeutic research laboratories, as well as more complex multimode plate readers more commonly used in core facilities and HTS facilities.

The assay is a red, competitive FI method (Figure 1). Because it is highly selective for ADP, the assay can be used with any enzyme that converts ATP to ADP, regardless of what other substrates are used. Examples of enzymes include protein, lipid, and carbohydrate kinases, ATPases, DNA helicases, carboxylases, and glutamine synthetase. (Source: BellBrookLabs)

Figure 1: *Schematic overview of the Transcreener® ADP² FI Assay.* (Source: BellBrookLabs)



The **Hidex Sense platereader** will help your lab become more effective. The touch screen user interface makes the operation safe and comfortable. Straightforward application focused operation minimizes time spent on instrument training, and is essential for superior results.

Figure 2. Hidex Sense platereader with a touchscreen user interface.

Materials required

Reader:

Hidex Sense multitechnology plate reader (425-301, -311)

Assay kit:

Transcreener® ADP^2 FI Assay Kit (3013-1K, BellBrookLabs)

Filters:

Excitation filter 560bw40 nm (Hidex code 1425-8560_40)
Emission filter 630bw40 nm (Hidex code 1425-8630_40)

Microplate:

Black Low volume, Round bottom 384 microplate (Corning Ref. 4514)

Kit components

- ADP Alexa594 Tracer, 800 nM
- ADP^2 Antibody-IRDye® QC-1, 1,56 mg/mL
- Stop & Detect Buffer B, 10X
- 5 mM ADP solution
- 5 mM ATP solution

Reader settings

Filters	Excitation 560/40 nm	Apertures	Excitation 1 mm
	Emission 630/40 nm		Emission 2 mm
	Dichroic mirror 600 nm	Flashes	100
Focus	11.2	Lamp power	Low

Assay protocol

- Dispense 10 µL of ATP/ADP dilution dilutions in 24 replicates
- Dispense 10 µL of free tracer and buffer blank in 12 replicates
- Add 10 µL of ADP Detection mix to all wells
- Incubate at RT for 1 hour
- Measure fluorescence intensity

Validation criteria

- Z'-Factor ≥ 0.7 at 10% conversion of 10 µM ATP
- Read time to achieve Z' specifications ≤ 5 minutes

The key instrument parameters for Transcreener® HTS assay performance were identified by running a

10 µM ATP/ADP standard curve (24 replicates), as standard curves of this type to mimic enzyme reactions. Starting with 10 µM ATP, ADP was added in increasing amounts and ATP is decreased proportionately, maintaining a total adenine nucleotide concentration of 10 µM. In order to validate an instrument for use with the Transcreener® FI Assays, a Z' > 0.7 at 10% conversion of 1 µM ATP within less than 5 minutes/304 wells was required.

Data analysis

The following equation to calculate the Z′ factor was used:

$$Z' = 1 - \frac{[(3 \times SD_{x\% \text{ conversion}}) + (3 \times SD_{0\% \text{ conversion}})]}{|(RFU_{x\% \text{ conversion}}) - (RFU_{0\% \text{ conversion}})|}$$

Results

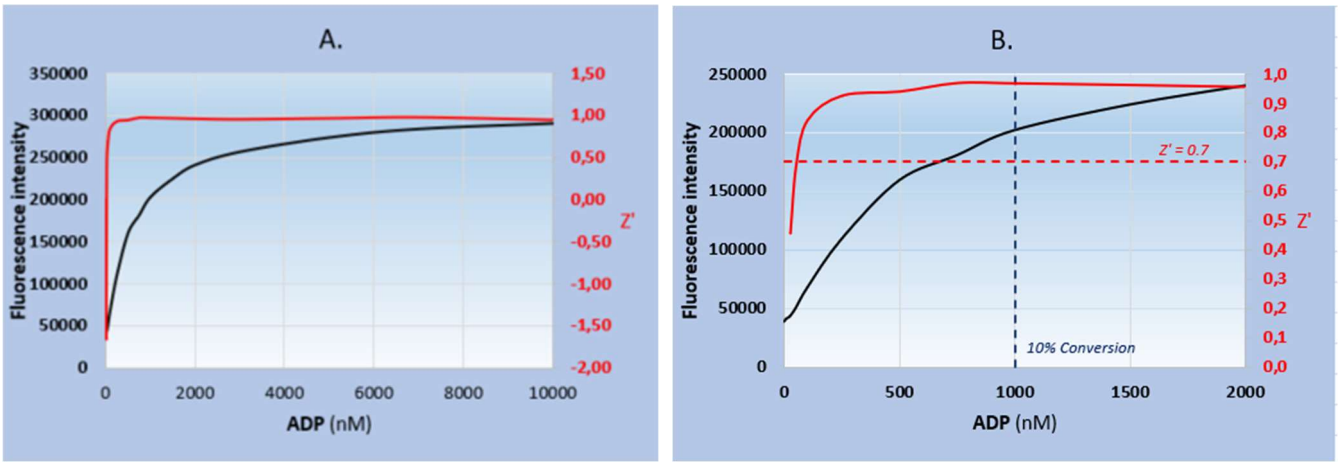


Figure 3. . A). Z' values (red line) and fluorescence intensities (black line) observed in a standard curve mimic conversion of 10 µM ATP to ADP. B). Zoomed view of the 0-2000 nM ADP section of the standard curve shows the Z' validation minimal qualification data (red dotted line) and 10% ATP conversion validation point (blue dotted line).

Conclusions

We can conclude that the Hidex Sense platereader gives an easy-to-use platform to perform Transcreener® FI assay. The Sense user-interface has a ready-made assay protocols which can be easily modified to be used with different assay setups. Using the optimized reader settings the total measurement time of a 384-well microplate is 108 seconds.

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