PHERAstar® *FSX* certified for Transcreener® assays

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- **Detect enzyme activity in HTS format**
- **PHERAstar® *FSX* equipped with Transcreener specific optic modules to measure signals of far-red dyes**
- **High sensitivity data with read times < 30 seconds for a whole 384-well microplate**

Introduction

The Transcreener® assays from BellBrook Labs offer a flexible approach to detect enzyme activity in high throughput screening (HTS) format. The assay is based on the direct detection of nucleotides such as ADP, GDP, UDP, GMP and AMP allowing determining the activity of e.g. methyltransferases, acetyltransferases, kinases or GTPases. The assays use far-red dyes and are available in three detection modes:

- Transcreener FP (Fluorescence Polarization)
- Transcreener FI (Fluorescence Intensity)
- Transcreener TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer)

In this application note we show the performance of the PHERAstar *FSX* microplate reader from BMG LABTECH in Transcreener® assays. In validation measurements the output values resulted in high sensitivity data while the read time per 384-well microplate can be < 30 sec. confirming the instrument to be an excellent option for HTS.

Assay Principle

After the enzymatic reaction ADP is present in the sample. A general description of the different kinds of ADP detection is presented in Fig. 1.

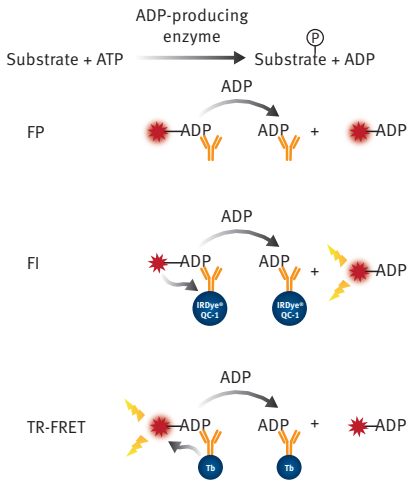

Fig. 1: Assay principle.

Transcreener FP principle
ADP-labeled with Alexa633 and conjugated to an antibody is added to the well. ADP that is produced during the enzymatic reaction will compete with the Alexa633-ADP for the binding site of the ADP antibody and will displace the Alexa633-ADP. This will result in a decreased FP value.

Transcreener FI principle
ADP-labeled with Alexa594 is conjugated to an antibody that carries a quencher. ADP that is produced during the enzymatic reaction will compete with the Alexa594-ADP for the binding site of the ADP antibody and will displace the Alexa594-ADP. That brings fluorescent dye and quencher into distance resulting in an increase in FI value.

Transcreener TR-FRET principle
ADP-labeled with acceptor dye HiLyte647 is conjugated to an antibody that carries donor terbium chelate. ADP that is produced during the enzymatic reaction will compete with the HiLyte647-ADP for the binding site of the ADP antibody and will displace the labeled ADP. That brings TR-FRET donor and acceptor out of proximity and leads to a decrease in TR-FRET signal.

Materials & Methods

- Transcreener® ADP² FP, FI, TR-FRET assay kits
- Black 384-well small volume, low binding plate (Greiner)
- PHERAstar *FSX* microplate reader

ADP/ATP standards were prepared to mimic an enzymatic reaction. 10 µM ADP and 10 µM ATP stock solutions were combined at varied proportions to create different percent conversions of ATP to ADP ranging from 0 to 10 µM. 10 µl of standard and 10 µl of ADP detection mixture (containing ADP antibody and ADP far red tracers) are combined in the microplate and incubated for 1 hour at room temperature. After incubation the plate was measured in the PHERAstar *FSX*.

PHERAstar FSX instrument settings

	Transcreener FP	Transcreener FI	Transcreener TR-FRET
Simultaneous Dual Emission	yes		yes
Optic module	FP 590 675 675	FI 580 620	TRF 337 665 620
Excitation source	Flash lamp	Flash lamp	Laser or flash lamp
			Int. start: 50 µs Int. time: 50 µs
Gain/Focus	Should be adjusted prior the measurement		

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Results & Discussion

Transcreener FP

Fig. 2 shows that with the PHERAstar *FSX* an assay window higher than 200 mP has been reached (assay window = values for 100 % ATP- values for 100 % ADP).

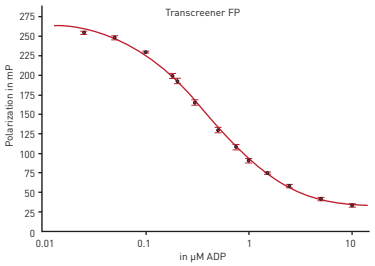

Fig. 2: 10 µM ADP standard curve of the Transcreener FP assay.

The effect of the number of flashes on robustness of measurement is shown in Table 1.

No. of flashes	100	50	25	13
Δ mP at 10 % conversion	156	157	158	158
Z' at 10 % conversion	0.878	0.819	0.780	0.749
Read time for a full 384w plate	3 min 31 sec	2 min 26 sec	1 min 53 sec	1 min 37 sec

The assay can be reliably measured in less than 2 min for a whole 384-well microplate.

Transcreener FI

Transcreener FI signals increase with increasing ADP concentrations. According to the certification requirements it is necessary to obtain a Z´ factor of 0.7 at 10 % ATP conversion. Fig. 3 shows that with the PHERAstar *FSX* this Z´factor is achieved at 4% conversion, much lower than the certification requirements.

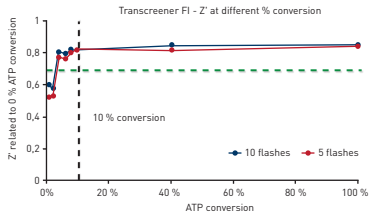

Fig. 3: Z´values obtained in a standard curve mimic conversion of 10 µM ATP to ADP.

Transcreener TR-FRET

TR-FRET measurements can be performed by either using the flash lamp or the laser in the PHERAstar *FSX*. A standard curve obtained with the laser is shown in Fig. 4.

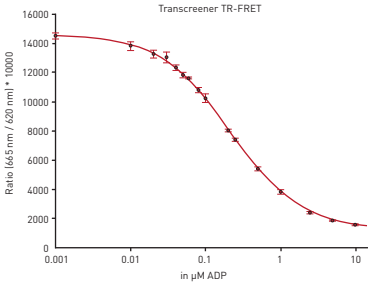

Fig. 4: 10 µM ADP standard curve of the Transcreener TR-FRET assay.

Flash lamp and laser give comparable data in terms of assay window and stability of data. The advantage of the laser is the use of a less number of flashes. In the flying mode the whole plate can be read in less than 30 sec (Table 2).

Flashes Flash lamp	Z' value	Read time	*Flahes Laser*	Z' value	Read time
50	0.815	2 min 27 sec	10	0.896	2 min 21 sec
25	0.807	1 min 53 sec	5	0.884	1 min 49 sec
10	0.747	1 min 33 sec	1	0.830	1 min 23 sec
5	0.746	1 min 26 sec	flying	0.736	27 sec

Validation criteria from BellBrook Labs

- 384-well format
- Z´- Factor ≥ 0.7 at 10 % conversion of 10 µM ATP
- Δ mP ≥ 95 mP at 10 % conversion of 10 µM ATP
- Read Times to achieve specifications ≤ 5 minutes

Conclusion

Based on the data shown the PHERAstar *FSX* has obtained all three Transcreener certifications.

PHERAstar® *FSX*

*The PHERAstar *FSX* is the newest PHERAstar reader.