

Evaluation of the Fusion Optics of Tecan's Spark™ 10M for HTRF® assay technology

The unique Fusion Optics enables the combination of filters and monochromators within one instrument for excellent performance in high throughput screening and the flexibility required for assay development.

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Introduction

HTRF (homogeneous time-resolved fluorescence) is a time-resolved fluorescence resonance energy transfer (TR-FRET)-based assay system from Cisbio Bioassays for the analysis of various molecular interactions and binding studies. Based on energy transfer between a long-lived europium or terbium cryptate donor and a variety of red or green acceptors, the assay combines the advantages of FRET and TRF.

HTRF assays place high sensitivity demands on multimode readers. Tecan's latest multimode reader, the Spark 10M, offers a number of features to meet these demands and ensure optimal assay performance, including the unique

Fusion Optics. Fusion Optics combine the sensitivity of filters and the flexibility of monochromators (MCRs) in one instrument, offering a choice of entirely filter- or MCR-based measurements, or simultaneous use of a combination of the two. Automated Z-position optimization and an integrated dichroic mirror that is perfectly suited to HTRF measurements further increase the Spark 10M's outstanding sensitivity. This poster describes the results of an in-depth validation of the Spark 10M reader for HTRF measurements using different Cisbio Bioassay kits, including the HTRF reader control kit (1) designed for performance validation of multimode readers, the Human TNFα assay kit (2), the cAMP HiRange assay kit (3) and the IP-One Tb assay kit (4).

Results

HTRF reader control kit

The Spark 10M delivers excellent results for the RCK using filters on the emission side. On the excitation side, both filters and MCRs can be used to meet Cisbio's acceptance criteria (6). Therefore, validation of the Spark 10M reader for HTRF was performed with both filter/filter and MCR/filter combinations. The measurement results demonstrate the suitability of the Spark 10M for HTRF analyses. The Spark 10M meets all the RCK performance criteria specified by Cisbio for white plates in both measurement modes and for black plates using filters only (Table 1).

Plate	Cisbio Criteria	Filter/filter		MCR/filter
		White	Black	White
% CV Std o	≤10	1.5	3.1	2.2
% CV Low	≤10	1.5	3.1	1.9
% CV High	≤10	1.3	1.4	1.4
ΔF Low (%)	≥15	41	37	40
ΔF High (%)	≥600	1,144	1,121	1,111
S/B	≥40	518	456	504

Table 1: Results of the RCK measured with 75 flashes. Comparison of the different reader combinations with white and black plates.

Human TNFα kit

The human TNFα kit is the most sensitive assay tested during the validation and therefore requires excellent reader sensitivity. According to the evaluation protocol provided, the detection limits for TNFα were calculated based on calibrators 1 to 3. They are below the 20 pg/ml required for the filter/filter combination with both black and white plates (Table 2).

Plate	Cisbio Criteria	Filter/filter		MCR/filter
		White	Black	White
Gain 620/665		158/223	197/255	184/248
% CV Std o		1.7	3.2	2.4
ΔF Cal 1 (%)		14	12	12
ΔF Cal 2 (%)		38	32	36
ΔF Cal 3 (%)		71	65	68
Linearity		0.9984	0.9998	0.9979
DL (pg/ml)	<20	4.39	10.59	7.03

Table 2: Example results of a TNFα measurement with filters only and the Fusion Optics (MF) using black and white plates.

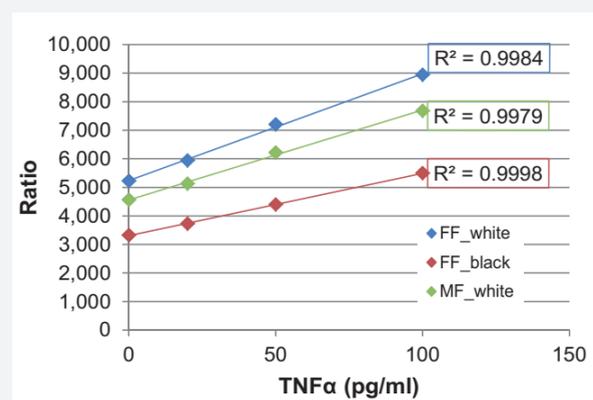


Figure 1: Linearity of the TNFα dilution series with filters only and Fusion Optics (MCR/filter) using black and white plates.

The Fusion Optics (MCR/filter) exceeds the Cisbio criteria with white plates (Table 2), but not with black plates (not shown). Therefore, following measurements using the Fusion Optics were performed with white plates only. Measurement of a TNFα dilution series showed an almost linear path ($R^2 \geq 0.9979$) for white (filter/filter and MCR/filter) and black plates (filter/filter only) between 20 and 100 pg/ml TNFα (Figure 1).

cAMP HiRange kit

As shown in Figure 2, the ΔF values obtained with the cAMP dilution series are inversely proportional to the cAMP concentration. This results in the sigmoidal curve that is typical of competitive assays. The detailed measurement results are summarized in Table 3. In each case a sufficient dynamic range with a signal-to-blank ratio of ≥20 was achieved, with EC₅₀ values of <25 nM. While white plates show equally good performance the ΔF values – and therefore the signal-to-blank ratios – are lower for black plates.

Plate	Cisbio Criteria	Filter/filter		MCR/filter
		White	Black	White
Gain 620/665		153/159	186/192	176/184
EC ₅₀ (nM)	<25	7.32	4.09	7.19
S/B	>20	31	20	30

Table 3: Example measurement results obtained with the cAMP HiRange kit using filters only and the Fusion Optics (MCR/filter) with black and white plates.

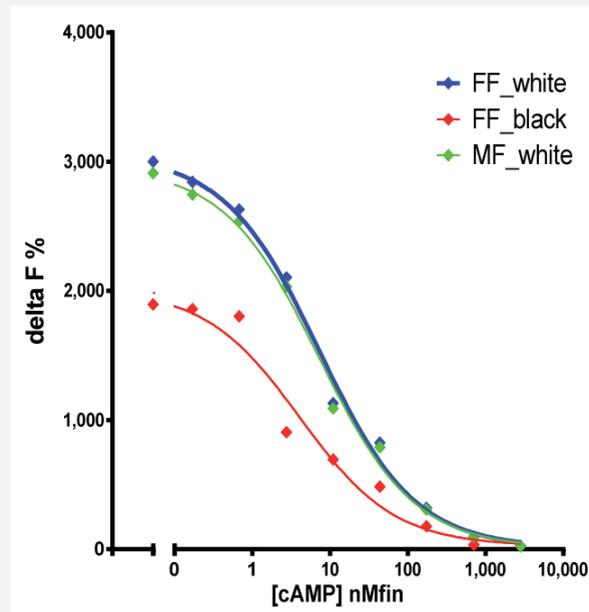


Figure 2: Competitive cAMP curves obtained with the filter/filter (black and white plates) and MCR/filter (white plate only) combinations.

IP-One Tb kit

The IP-One Tb kit was used to analyze the performance of the Spark 10M for measurement of a terbium cryptate donor in combination with a red acceptor. The Cisbio performance criteria were met for both filter/filter and MCR/filter measurements using white plates, and for filters only using black plates (Table 4). Figure 3 shows typical IP1 standard curves. The best performance was achieved using white plates and the filter/filter combination. All other measurements resulted in reduced ΔF, signal-to-blank ratio and EC₅₀ values.

Plate	Cisbio Criteria	Filter/filter		MCR/filter
		White	Black	White
S/B		28.1	22.8	24.5
EC ₅₀ (nM)	<150	122	132	138

Table 4: Example of measurement results for the IP-One Tb kit using the filter/filter (black and white plates) and the MCR/filter (white plate only) combinations.

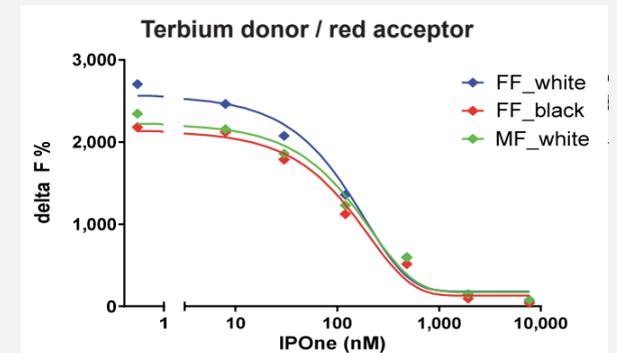


Figure 3: Competitive assay curves for IP1 concentration obtained with the filter/filter (black and white plates) and MCR/filter (white plate only) combinations.

cAMP green reader control kit

The cAMP green reader control kit uses a terbium anti-cAMP conjugate coupled to a cAMP green acceptor. It was used to analyze the Spark 10M's ability to measure HTRF with a terbium-donor and green acceptor. All evaluated combinations of the Fusion Optics met the criteria defined by Cisbio (Table 5). However, the signal-to-blank ratio and EC₅₀ values using black plates are slightly reduced compared to measurements with the white plate, regardless of the Fusion Optics combination used. This is also evident in the competitive assay curves, which show decreased ΔF values using the black plates (Figure 4).

Plate	Cisbio Criteria	Filter/filter		MCR/filter
		White	Black	White
S/B		47.7	41.4	47.4
EC ₅₀ (nM)	<75	65	62	64

Table 5: Example measurement results for the cAMP coupled to green acceptor using the filter/filter (black and white plates) and MCR/filter (white plate only) combinations.

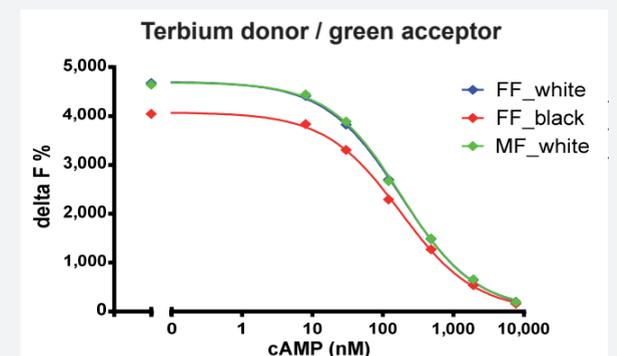


Figure 4: Competitive assay curves for cAMP concentration with a terbium donor and a green acceptor. The measurements were performed using the filter/filter (black and white plates) and the MCR/filter (white plate only) combinations.

Material and methods

- Spark 10M multimode reader (Tecan, Austria)
- 384-well, small volume microplates, black and white (Greiner® Bio-One, Germany)
- HTRF reader control kit (Cisbio Bioassays, France)
- cAMP HiRange assay kit (Cisbio Bioassays, France)
- Human TNFα assay kit (Cisbio Bioassays, France)
- IP-One Tb assay kit (Cisbio Bioassays, France)
- cAMP-green reader control kit (internal control; not commercially available. Cisbio Bioassays, France)

Assay protocols

All kits were used according to the manufacturer's instructions (1-4). For validation of the terbium donor/green acceptor configuration, cAMP directly coupled to a green acceptor was used in combination with an anti-cAMP antibody coupled to a terbium donor. Each kit was measured three times in 384-well small volume

plates (white and black) using a filling volume of 20 µl per well with optimized measurement parameters (5). The Spark 10M was validated using the filter/filter combination (FF), as well as the Fusion Optics with MCR on the excitation side and the filters on the emission side (MF).

Conclusion

The innovative Spark 10M multimode reader takes advantage of Tecan's unique Fusion Optics to provide customers with optimized solutions, allowing for the first time to combine filters and monochromators within one measurement. Using filters for both, excitation and emission, the system delivers excellent results for black or white plates. It also meets all the criteria required to successfully perform HTRF measurements in white plates using MCRs on the excitation side and filters on the emission side. These combinations can therefore be regarded as HTRF certified. In addition to kits with a europium donor, reliable, sensitive quantification can also be performed using a terbium donor with both red and green acceptors.

References

- (1) Manual Reader Control Kit (http://www.cisbio.com/sites/default/files/resources/htrf_rck.pdf)
- (2) Manual cAMP HiRange Kit (http://www.cisbio.com/sites/default/files/resources/cisbio_dd_pi_62AM6PEB.pdf)
- (3) Manual Human TNFα kit (http://www.cisbio.com/sites/default/files/resources/cisbio_dd_pi_62TNFPEB.pdf)
- (4) Manual IP-One Tb kit (http://www.cisbio.com/sites/default/files/resources/cisbio_dd_pi_62IPAPEB-62IPAPEC-62IPAPEJ.pdf)
- (5) Application Note: Homogeneous time-resolved fluorescence (HTRF®) assay technology in the Spark™ 10M multimode reader. 398768 V1.0. 05-2015
- (6) Technical Note: The ingenious Fusion Optics in the Spark 10M multimode reader. 398568 V1.0. 12-2014

Abbreviations

- HTRF Homogeneous Time-Resolved Fluorescence
- TRF time-resolved fluorescence
- FRET fluorescence resonance energy transfer
- MCR monochromator
- RCK reader control kit
- cAMP cyclic adenosine 3'-5'-monophosphate
- IP1 inositol monophosphate
- TNFα tumor necrosis factor-alpha

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