HTRF® measurement with the Hidex Sense Platereader

Background

HTRF® (homogeneous time resolved fluorescence) is a technology based on TR-FRET (time-resolved fluorescence resonance energy transfer) chemistry. TR-FRET unites standard FRET chemistry with the use of lanthanide donors, fluorophores with long emission half-lives. This powerful combination provides significant benefits to drug discovery researchers including assay flexibility, reliability, increased assay sensitivity, higher throughput and fewer false positive/false negative results. While HTRF® is based on TR-FRET chemistry it has many properties that separate it from other TR-FRET products. These include the use of a lanthanide with an extremely long half-life (Europium), conjugation of Eu^{3+} to cryptate, an entity which confers increased assay stability and the use of a patented ratiometric measurement that allows assay interference correction. Other HTRF® technology features include:

Features

- Homogeneous Assay Format
- Low Background
- Simplified Assay Miniaturization
- Low Compound & Media Interference
- Tolerant of Assay Additives such as DMSO & EDTA
- Cell-based Functional Assays

CISbio offers a reader validation kit to evaluate microplate reader performance and to use as instrumentation QC tool. This kit was read with the Hidex Sense multitechnology platereader.

Materials required

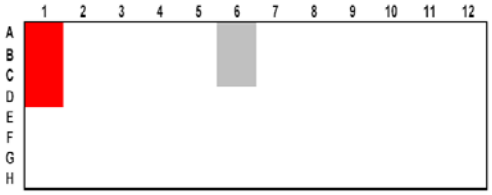
Hidex Sense multitechnology plate reader with time-resolved fluorescence function.
HTRF® Reader control kit (Prod. Code 62RCLPEA, Cisbio Bioassays)
425-2330 (330bw80nm excitation filter)
425-3620 (620bw10 nm Donor emission filter with UV suppression filter)
425-3665 (665bw7.5 nm Acceptor emission filter with UV suppression filter)
Black Half-area 96-well microplate

Assay protocol

Test of the S/B with the 620 nm calibrator

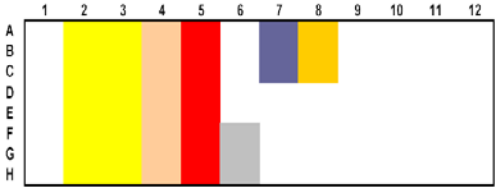
Reconstitute the 620 nm control with the diluents.
Leave this control at least for 30 minutes at room temperature before dispensing.
Dispense the control as indicated in the plate map below (A1-D1). A buffer blank will also be run in triplicate (A6-C6).

A1-D1: 620 nm control :
50µL of 620 nm control & 50 µL of reconstitution buffer
A6-C6: Buffer Blank :
50 µL of diluent & 50 µL of reconstitution buffer



Control of the detection limit

Dispense the reagents as indicated in the plate map below:



A2-H3: Standard 0 (Cryptate and XL665 conjugates diluted in reconstitution buffer: no FRET):
50 µL of diluent, 25 µL of Cryptate conjugate & 25 µL of XL665 conjugate

A4-H4: Low control (Cryptate and XL665 conjugates with low calibrator: low FRET):
50 µL of low calibrator, 25 µL of Cryptate conjugate & 25 µL of XL665 conjugate

A5–H5: High control (Cryptate and XL665 conjugates with high calibrator: maximum FRET):
50 µL of high calibrator, 25 µL of Cryptate conjugate & 25 µL of XL665 conjugate

F6-H6: Buffer Blank:
50 µL of diluent & 50 µL of reconstitution buffer

A7-C7: XL665 blank (XL665 conjugate diluted in reconstitution buffer):
50 µL of diluent, 25 µL of XL665 conjugate & 25 µL of reconstitution buffer

A8-C8: Cryptate blank (Cryptate conjugate diluted in reconstitution buffer):
50 µL of diluent, 25 µL of Cryptate conjugate & 25 µL of reconstitution buffer.

Incubate the plate for 18 hours at room temperature.

Data reduction

Means and CV's are calculated for each set of samples and for both wavelengths (665 nm and 620 nm). The ratio R (665 nm/620 nm) is calculated from this data as follows:

$$R = \frac{cps_{665nm}}{cps_{620nm}} \times 10{,}000$$

From this ratio, Delta R and Delta F are calculated for both the low and high controls. Delta R is given by the formula:

$$\text{Delta R} = R_{CalX} - R_{Std0}$$ Cal X is either the low or the high calibrator

Delta F, which represents the S-B/B, can then be obtained with the formula:

$$\text{Delta F} = \frac{\text{Delta R}}{R_{Std0}}$$

XL665 and Cryptate blanks are only used if the instrument fails to reach the norms. In such a case, this data will help to determine the reason for the failure.

Reader settings

Filters	Excitation 330/80 nm
	Emission Donor 620/10 nm
	Emission Acceptor 665/7.5 nm
	Mirror 400 nm
Window	Delay 200 µs
	Length 100 µs
Aperture	Excitation 8 mm
	Emission Donor 8 mm
	Emission Acceptor 8 mm
Flashes	100

Results

The counts are displayed in the data tables with calculations according to the formulas defined in chapter data reduction.

Test of the S/B with the 620 nm calibrator

	1	2	3	4	5	6
A	5332					45
B	5009					41
C	4806					42
D	4934					Avg. 43
E	Avg. 5020					

Control of the detection limit

Emission Donor 616 nm

	1	2	3	4	5	6	7	8
A		4040	4094	3946	4047		33	3902
B		3919	3953	3960	3669		43	4086
C		3870	3901	3859	3890		41	3786
D		3909	3967	3996	3832			
E		3812	3541	4001	3648			
F		3836	3849	3805	3660	53		
G		3935	3815	3883	3744	29		
H		3635	3875	3797	3504	45		

Emission Acceptor 665 nm

	1	2	3	4	5	6	7	8
A		48	59	73	606		10	51
B		55	61	71	604		15	43
C		59	48	76	586		14	50
D		56	53	65	592			
E		59	50	66	576			
F		52	51	91	513	7		
G		55	50	80	550	13		
H		63	53	58	552	9		

Ratio

	1	2	3	4	5	6	7	8
A		119	144	185	1497		3030	131
B		140	154	179	1646		3488	105
C		152	123	197	1506		3415	132
D		143	134	163	1545			
E		155	141	165	1579			
F		136	133	239	1402	1321		
G		140	131	206	1469	4483		
H		173	137	153	1575	2000		

Ratio (Avg.)

	1	2	3	4	5	6	7	8
A		Standard 0		Low	High		XL665	K
B				control	control		blank	blank
C		141		186	1527		3311	123
D								
E								
F						Buffer		
G						blank		
H						2601		

Delta R (Avg.)

	1	2	3	4	5	6	7	8
A				Low	High			
B				control	control			
C				45	1387			
D								

Delta F (Avg.)

	1	2	3	4	5	6	7	8
A				Low	High			
B				control	control			
C				0.319	9.84			
D								

Summary of results and acceptance criteria (norm)

	665 nm		620 nm		Norm
	Mean	%CV	Mean	*%CV*	
Buffer blank	10	33.3	5020	*4.5*	
620 nm control	74	11.8	43	*4.9*	
S/B			118		≥ 40

	DF %	Norm
Low calibrator	32	≥ 15 %
High calibrator	984	≥ 600 %

	%CV ratio	Norm
Standard 0	*9.4*	≤ 10 %

	665 nm		620 nm	
	Mean	*%CV*	Mean	*%CV*
Buffer blank	10	*31.6*	42	*28.9*
Cryptate blank	48	*9.1*	3925	*3.9*
XL665 blank	13	*20.4*	39	*13.6*

Conclusions

The quality control sheet shows all criteria defined by CISbio and values are indicating that Hidex Sense suits well for high throughput screening using this popular and powerful technology.

Reference

1. HTRF® Reader control kit insert

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