

Artemis: the new HTRF[®] reader designed for the life science field

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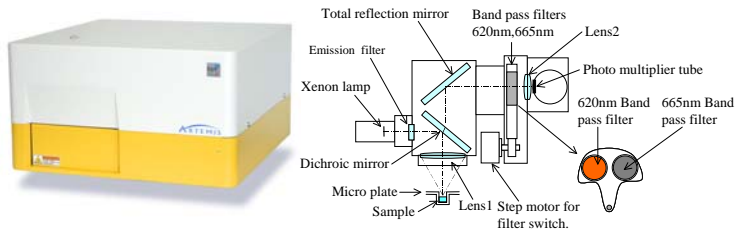
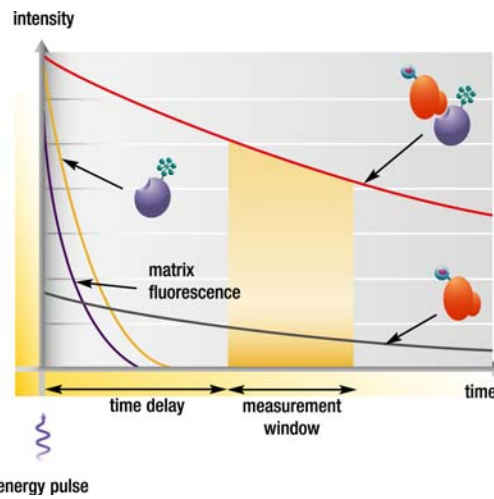


Fig.1 "Artemis" and structure of an optics unit.

HTRF® is a highly efficient immunoassay method: The washing procedure is unnecessary and the assay uses a patented measurement ratio which can compensate for the presence of colored compound interferences in the assay. "Artemis" was developed to meet life science research needs for HTRF® applications.

We have developed a new reader, the Artemis, dedicated to HTRF® : It features a highly stable Xenon flash lamp, low stray light and internal reflection optical unit, well optimized filters and software that are specific for HTRF®. We adopted the Xenon flash lamp that emits light as strong ultraviolet rays, the emission system is simple, and large-scale power supply is not needed. Fig.1 shows the exterior appearance and an optical unit. With CIS bio international we have checked the optimal reading conditions to reach the best possible results with HTRF®. The parameter involved were **Delay time**, **Integral time**, and **Flash times**. We finally compared the performance of an optimized "Artemis" with other HTRF® compatible readers.



The principle of HTRF® is shown in Fig2: When two entities come close enough to each other, excitation of the long-lived donor (cryptate-labeled) by an energy source (e.g. flash lamp fitted with a suitable filter) triggers an energy transfer towards the acceptor (XL665) via FRET. Usually short lived fluorescence, this acceptor then emits specific long-lived fluorescence at a given wavelength.

Fig.2 TR-FRET format

The energy pulse from the excitation source (flash lamp, laser) is followed by a time Delay, allowing interfering short-lived fluorescence (compounds, proteins, medium...) to decay. The readout must be performed at 665nm and 620nm with a time-resolved module which enables the introduction of the necessary delay between the excitation flash and the fluorescence measurement. (Time-resolved readout).

MATERIALS AND METHODS

"Artemis" was used for the measurement of HTRF®.

- Reader Control kit(Ref#62RCLPEA; Reader control kit a straightforward procedure for reader checks.)
- TNF alpha kit (Ref#62TNFPPEB; To draw a standard curve of a sandwich immunoassay.)
- Cyclic AMP dynamic 2 kit (Ref#62AM4PEB; To draw a standard curve of a competitive immunoassay.)
- Plate a with Greiner 384-well small volume black plate (Ref# 784076) and Corning 384-well small volume white plate (Ref#3674).

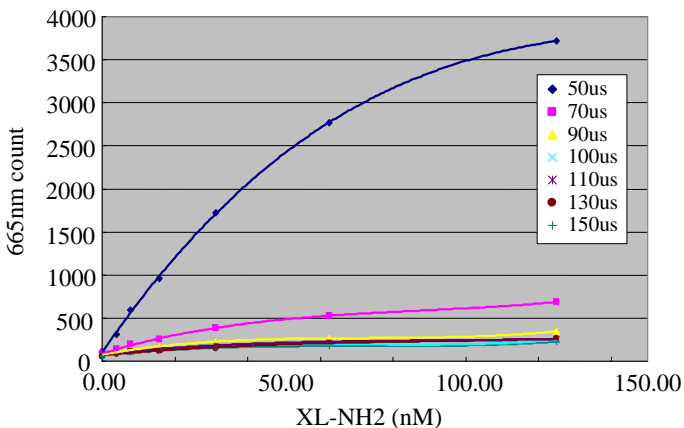
HTRF® Acquired data calculated, and signal ratio and DF values in the measurement analyzed by the calculation type below:

Signal ratio = (665nm / 620nm) X 10000

DF values (%) = [(ratio of sample - ratio of blank)/ratio of blank] X 100

RESULTS & DISCUSSION

Optimization of "Artemis"



delay 50 - 150us, Integral 400us, Flash 40 384-Black Plate

Fig.3 Effects of Delay time

In this study, the delay time was optimized: The purpose of the optimization of Delay Time is to exclude the influence of short-lived fluorescence. The short-lived fluorescence is mainly emitted from the proteins etc. contained in XL665 and the sample. Delay time optimization means finding the time needed to enable XL665 short-lived fluorescence to decay even at high concentrations. The concentrations of Free XL665 selected were 0, 3.91, 7.81, 15.63, 31.25, 62.50, and 125.0(nM). Delay times of 50, 70, and 90,100,110,130,150 μ s were set, and we measured the levels. Fig.3 shows the results. We clearly confirmed the increase in the number of counts for 50 μ s, 70 μ s, and 90 μ s. The counts became constant at a low level after 100 μ s or more. As a result, 100 μ s was assumed to be an optimal value.

In this study, the optimization of Integral time was carried out by using the HTRF® Reader Control kit. The purpose was to obtain the highest possible DF values. Integral time was optimized using the Low control, High control, and Standard 0 of the HTRF® reader Control kit. Integral times were set and measured at 100,200,400,600(μ s). The results are shown in Table1. We found the Integral time of 100 μ s to be the optimal value, as the DF values were the highest.

Table 1. Effects of Integral time on DF%

Integral time [μ s]	100	200	400	600
Std.0 (n=20)	0%	0%	0%	0%
Low Control (n=10)	104%	89%	79%	81%
High Control (n=10)	2228%	1886%	1756%	1748%

delay 100, Integral 100-600us, Flash 50 384-White Plate

Table 2. Effects of Flash times on CV%

Flash No.	Black				White				(Norms)
	25	50	100	200	25	50	100	200	
Std.0 (n=20)	8.0%	5.1%	3.6%	3.1%	2.7%	2.0%	1.8%	1.2%	<10%
Low Control (n=10)	4.8%	3.3%	4.0%	1.8%	3.3%	2.3%	2.3%	2.4%	
High Control (n=10)	2.0%	2.3%	1.6%	0.9%	1.1%	0.4%	0.7%	0.5%	
Analysis time (sec)	355	650	1100	1968	355	650	1100	1968	

delay 100us, Integral 100us, Flash 25-200 384-White or Black Plate

The optimal value of Flash times is verified: In this study, a Flash number was optimized by using the HTRF® Reader Control kit. The optimization of Flash times means obtaining a good compromise between low CV value and a short measurement time. When Flash times increases, measurement time becomes longer but it lowers CVs. Moreover, the CV values are influenced by the plate color. Therefore, we optimized respectively for white plates and black plates. The reagents used were Low control, High control, and Standard 0 of the kit. Flash times were set and measured at 25 and 50,100,200. The results are shown in Table2. The CV values for a black plate are low for Flash times 100 and 200. We judged that measurement time was too long at 200, and assumed Flash time of 100 to be an optimal value. The CV value of a white plate is low in all conditions. We assumed a Flash times of 50 to be an optimal value given the stability of measurements at 25. However, to simplify set up for user, CIS bio international recommends the 100 for both black and white plates.

Comparison between Artemis and the other companies product

TNFα immunoassay standard curves

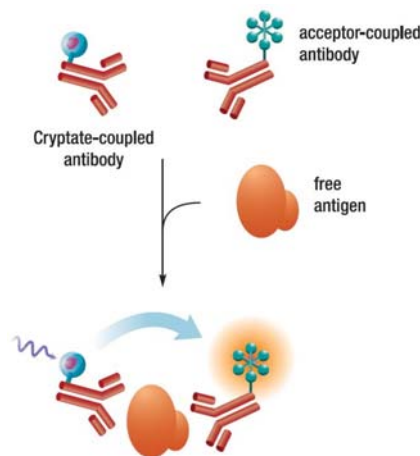


Fig.4 Sandwich immunoassay format

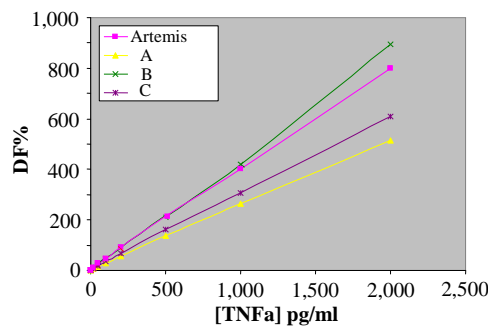


Fig.5 TNF α standard in diluent in white plate

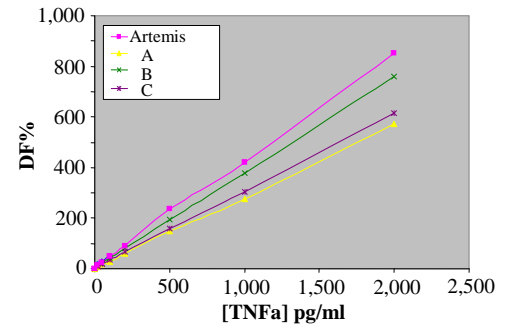


Fig.6 TNF α standard in diluent in black plate

HTRF® TNF α sandwich immunoassay uses two anti- TNF α antibodies labeled respectively with Eu3+ Cryptate and XL665. FRET signal intensity is proportional to the TNF α-antibody complexes formed and therefore to TNF α concentration (Fig 4).The plates used were 384-well small volume white and black.The kit was read on the Artemis using the set up previously optimized. (**Delay time 100,Integral time 100,Flash times 50 for white plate and 100 for black plate**). The plates were run on several HTRF® compatible readers (Fig.5, 6).

cAMP immunoassay standard curves

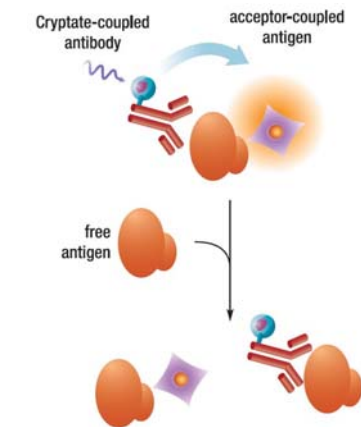


Fig.7 Competitive immunoassay format

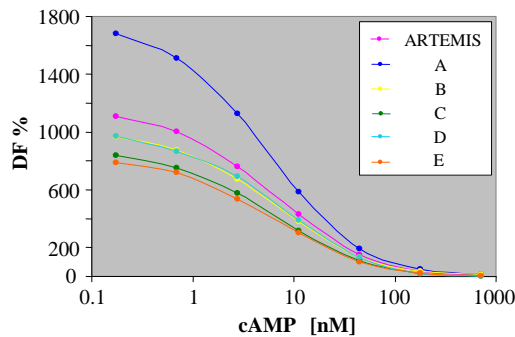


Fig.8 cAMP in 384 well white white plate

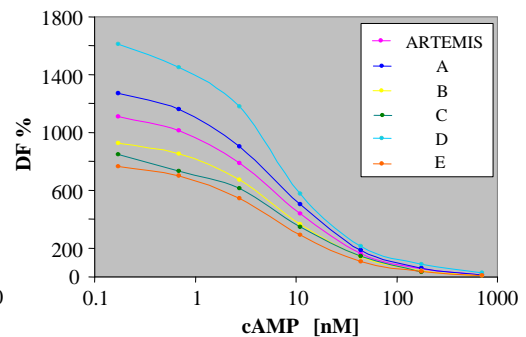


Fig.9 cAMP in 384 well white Black plate

HTRF® cAMP competitive immunoassay uses Eu3+ Cryptate-labeled anti cAMP antibody and d2 labeled cAMP. The cAMP present in the sample competes with d2-labeled cAMP for binding to and Eu3+ Cryptate-labeled anti-cAMP antibody. FRET signal is therefore inversely proportional to cAMP concentration (Fig.7). The plates used were 384-well small volume white and black.The kit was read on the Artemis using the set up previously optimized. (**Delay time 100, Integral time 100, Flash times 50 for white plate and 100 for black plate**). Results are shown in Fig.5, 6. The Artemis performance is in harmony with the other microplate readers. "Artemis" gave the best detection limit for such an assay (Table 3) and showed good performances compared with the other microplate readers.

Table3. detection limit

	Artemis	A	B	C	D	E	Unit
Black plate	0.140	0.210	0.237	1.899	0.294	0.271	(nM)
White plate	0.318	0.286	0.364	0.346	0.326	0.369	(nM)

CONCLUSION

The optimal settings for "Artemis" are Delay time 100, and Integral time 100. Flash times can be set at white plate 50, black plate 100 (or both, at 100). Performance comparisons using TNF α and cAMP showed highly satisfactory results compared with other HTRF® compatible readers. The features demonstrated above are convincing arguments for "Artemis" to be the HTRF® compatible of choice for life science research laboratories. Performance unites with user-friendliness and reliability !

Artemis



Product specification


Measuring method	Homogeneous Time-Resolved Fluorescence
Light source	High-output xenon flash lamp
Detection system	2 wavelength (620nm, 665nm) continuous measurement
Detection performance	Cis Bio International's standards to be met
Micro plate formats	96 Well (normal, half well) 384 Well (normal, small volume)
Reading time	For 20 Flash times 150sec(96Well)、350sec(384Well) For 50 Flash times 175sec(96Well)、650sec(384Well) For 100 Flash times 300sec(96well)、1100sec(384Well)
Computer interface	USB1.1 shielded (Less than 3m (9.8ft) in length)
Ambient conditions	indoor Temperature in operation +10 to +35°C (50 to 95 deg F) storage -10 to +50°C (14 to 122 deg F) humidity 20 – 80% (without condensation) atmospheric pressure
Dimensions	W440mm × L450mm × H250mm (17.3 inch) (17.7 inch) (9.8 inch)
Weight	maximum 14 kg (30.9 lb) approx.
Power supply	100/115/230 VAC ± 10% 47 – 63 Hz 70VA or less
Fuse rating	2A 100V/115V/230V
Applicable standards	CE、cTUVus

※This specification might change without a previous notice.

“Artemis” Manufacturing origin



2968-3, Ishikawa-cho Hachioji, Tokyo 192-0032 Japan

 Reagent manufacturing sales



BP84175 30204 Bagnols-sur-Ceze Cedex - France

“Artemis” Exclusive distributor except Japan



COSMO BIO CO., LTD.
Inspiration for Life Science

Toyo-Ekimae Bldg., 2-20, Toyo 2-Chome, Koto-ku,
Tokyo 135-0016 Japan

“Artemis” Japan sales agency

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