

MANUAL

Technology: HTRF™

HTRF Reader Control Kit

Part number

62RCLPEA

Storage: 2-8°C

Version: 08

Date: January 2026

BACKGROUND AND INTENDED USE

The HTRF™ reader control kit is designed for the calibration of all HTRF™ compatible readers and for validation of their ability to read HTRF™ technology. It allows their overall performances as well as the lifetime of their excitation source to be followed up through the actual counting rates of the reader. This kit is therefore intended to check and validate the optical and software configurations of readers, in particular when an upgrade has been installed. It may also be used for reader comparisons.

PROTOCOL

Reagents supplied and reconstitution

This kit includes the following reagents and components necessary to run one Revvity 96-w low volume assay plate:

Supplied reagents	Reagent reconstitution (stock solutions)		Working solutions
Europium Cryptate conjugate	1 vial lyophilized*	➡	Add 2.5 mL of reconstitution buffer to each vial. Mix gently.
XL665 conjugate	1 vial lyophilized*		
Low calibrator	1 vial lyophilized*	➡	Reconstitute each calibrator with diluent. See label indications for reconstitution volume. Mix gently after reconstitution.
High calibrator	1 vial lyophilized*		
620 nm control	1 vial lyophilized*		
Diluent (Fetal calf serum)	1 vial lyophilized*	➡	Add 5 mL of reconstitution buffer. Mix gently.
Reconstitution buffer 50 mM Phosphate, pH 7.0, 0.4 M KF	1 vial of 16 mL		
White Revvity 96-w low volume plate (Cat#66PL96001)	1 plate		

* Lyophilized in 50 mM phosphate buffer, pH 7, containing BSA free and stabilizers.

Allow the reagents to warm up to room temperature for at least 30 mins before reconstitution.

Reagent storage and stability

All reagents should be stored at 2-8°C until reconstituted. Under proper storage conditions, they are stable until the expiration date indicated on the labels. Reconstituted reagents, except the 620 nm control, are stable for 4 days at 2-8°C and they can be refrozen and thawed once.

After reconstitution the 620 nm control is stable for up to two weeks at room temperature. Do not freeze.

Assay protocol

The kit may be handled at room temperature with two different incubation times in order to permit two types of validation:

- Validation for multiple readers: in this case, an 18 hour incubation is carried out in order to perform multiple validations using the same assay plate. Following the 18 hour incubation, the signal is stable for 24 hours.
- Validation for single reader (e.g. after upgrade): in this case, a 3 hour incubation is carried out when the check for an upgraded instrument must be performed within ½ day. The assay should be read after 3 hours precisely. The signal is stable for another 30 mins.

The test consists of two steps. The first one aims at evaluating the Signal/Blank (S/B) ratio evaluation using the 620 nm calibrator. If the instrument passes this first test, the detection limit and the S/B of the complete assay are then evaluated.

Test of the S/B with the 620 nm calibrator

- Reconstitute the 620 nm control with the diluent
- Leave this control at least 30 mins 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 : 10 µL of 620 nm control & 10 µL of reconstitution buffer

(A6 - C6) : Buffer Blank : 10 µL of diluent & 10 µL of reconstitution buffer

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

- Read the plate

The S/B value is calculated using the formula:

$$\frac{S}{B} = \frac{\text{cps 620 nm}_{\text{control}}}{\text{cps 620 nm}_{\text{buffer blank}}}$$

S/B will have to be superior to the norms defined in the quality control datasheet. If the minimum value is reached, proceed with the second part of the assay.

Control of the detection limit

- Reconstitute conjugates, high and low calibrators
- Dispense the reagents as indicated in the plate map below

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

(A2 - H3) : Standard 0 (Cryptate and XL665 conjugates diluted in reconstitution buffer : no FRET) : 10 µL of diluent, 5 µL of Cryptate conjugate & 5 µL of XL665 conjugate

(A4 - H4) : Low control (Cryptate and XL665 conjugates with low calibrator : low FRET) : 10 µL of low calibrator, 5 µL of Cryptate conjugate & 5 µL of XL665 conjugate

(A5 - H5) : High control (Cryptate and XL665 conjugates with high calibrator : maximum FRET) : 10 µL of high calibrator, 5 µL of Cryptate conjugate & 5 µL of XL665 conjugate

(F6 - H6) : Buffer Blank : 10 µL of diluent & 10 µL of reconstitution buffer

(A7 - C7) : XL665 blank (XL665 conjugate diluted in reconstitution buffer) : 10 µL of diluent, 5 µL of XL665 conjugate & 5 µL of reconstitution buffer

(A8 - C8) : Cryptate blank (Cryptate conjugate diluted in reconstitution buffer) : 10 µL of diluent, 5 µL of Cryptate conjugate & 5 µL of reconstitution buffer

- Incubate the plate 3 hours or 18 hours at room temperature.

Important: if the 3-hour incubation is performed, the readout must be done within 30 minutes following the incubation (i.e. assay is not yet at equilibrium). If the 18-hour incubation is performed, the signal will be stable for up to 24 hours.

DATA REDUCTION

Reader set up

Hardware and software configurations will have to be in conformity with the setup defined by the manufacturer.

Calculation

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

$$\text{Ratio} = \frac{\text{cps}_{665\text{nm}}}{\text{cps}_{620\text{nm}}} \times 10,000$$

From this ratio, Delta Ratio and Delta F are calculated for both the low and high controls.

Delta R is given by the formula :

$$\text{Delta R} = R_{\text{CalX}} - R_{\text{Std0}}$$

(where 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 :

$$\text{DeltaF} = \frac{\text{Delta R}}{R_{\text{Std0}}} \times 100$$

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.

Norms

Reference values (norms) for all these measurements were established by common agreement between the instrument manufacturer and Revvity. Values are specified on the quality control data sheet attached page 4 (Appendix).

Quality control report

A quality control datasheet is supplied in the kit for each validation. This report contains a minima the information listed below:

- Serial number of the reader
- Instrument location (optional)
- Date of the test
- Batch number of the HTRF™ reader control kit
- 620 nm control value in comparison with reference value
- S/B value in comparison with reference value
- Delta F obtained on low calibrator in comparison with reference value
- Delta F obtained on high calibrator in comparison with reference value
- CVs obtained on standard 0 values in comparison with reference value
- Buffer blank value
- Cryptate blank value
- XL665 blank value
- Attached instrument set up (file window or snapshots)

REACH European regulations and compliance

This product and/or some of its components include a Triton concentration of 0.1% or more and as such, it is concerned by the REACH European regulations.

We recommend researchers using this product to act in compliance with REACH and in particular: to only use the product for in vitro research in appropriate and controlled premises by qualified researchers, ii) to ensure the collection and the treatment of subsequent waste, and iii) to make sure that the total amount of Triton handled does not exceed 1 ton per year.

Quality control datasheet

HTRF™ reader control kit - Appendix

Reader:

Reader serial number:

Instrument location (optional):

Date of test:

Batch number of HTRF™ reader control kit:

Incubation time:

Instrument set up (join file or window snapshots) :

	665 nm		620 nm		Norm
	Mean	CV %	Mean	CV %	
Buffer blank					
620 nm control					
S/B					≥ 40

Incubation time: ☐ 3 hours ☐ 18 hours

	DF%	Norm		DF%	Norm
Low calibrator		≥ 10 %			≥ 15 %
High calibrator		≥ 450 %			≥ 550 %

	CV% ratio	Norm
Standard 0		≤ 10 %

	665 nm		620 nm	
	Mean	CV %	Mean	CV %
Buffer blank				
Cryptate blank				
XL665 blank				



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