VIRAL DOUBLE-STRANDED RNA DETECTION KITS

PROTOCOL

Part # 64RNAPEG & 64RNAPEH

Test size#: 500 tests (64RNAPEG) and 10,000 tests (64RNAPEH) - assay volume: 20 µL

Revision: 05-Aug. 2021

Store at: -60°C or below (64RNAPEG); -60°C or below (64RNAPEH)

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of viral double-stranded RNA (dsRNA) in cell lysates of mammalian cells infected by a virus and offers a simple alternative to Quantitative PCR (qPCR).

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, dsRNA is detected in a sandwich assay by using anti dsRNA antibody labeled with Europium cryptate (donor), and anti dsRNA antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the dsRNA concentration.

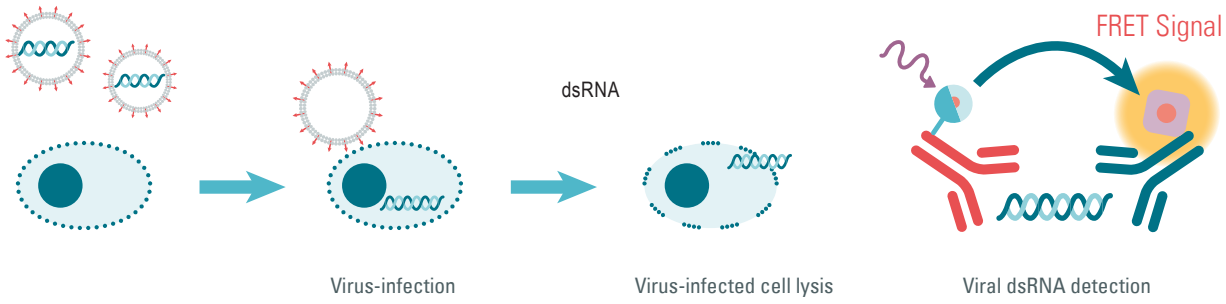
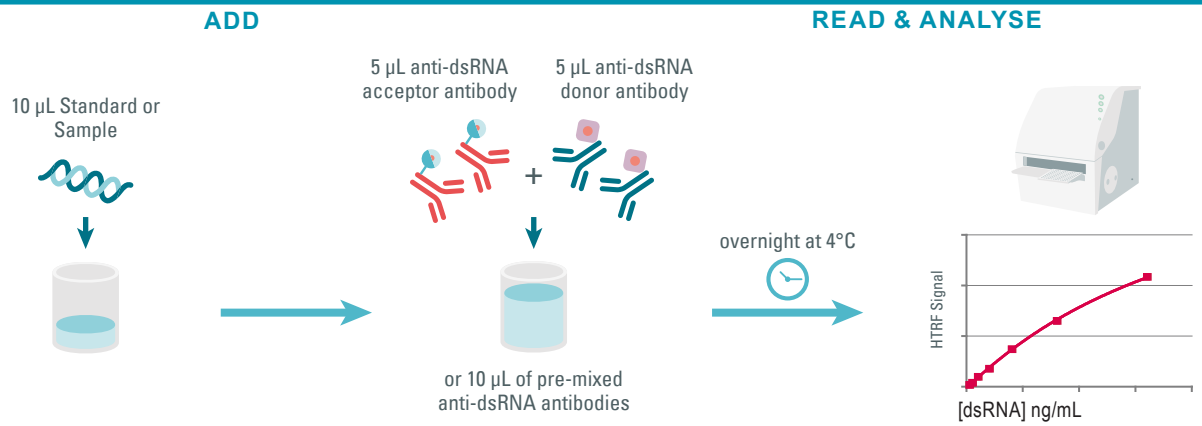


Figure 1: Principle of HTRF dsRNA sandwich assay.

PROTOCOL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

PerkinElmer® For the Better | cisbio

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 64RNAPEG	10,000 TESTS * CAT # 64RNAPEH
dsRNA Standard Frozen	1 vial - 50 µL 5 µg/mL	1 vial - 50 µL 5 µg/mL
dsRNA Eu Cryptate Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
dsRNA d2 Antibody	1 vial - 50 µL Frozen - 50X	1 vial - 1 mL Frozen - 50X
Lysis buffer #4 ** 4X	4 vials 2 mL	1 vial 130 mL
Detection buffer *** ready-to-use	1 vial 7 mL	1 vial 105 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume..
Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.
** Medium like cell culture medium can be an alternative to the diluent.
*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.cisbio.com/compatible-readers

- Small volume (SV) detection microplates - Use white plate only.

For more information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

STORAGE AND STABILITY

Reagents

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Detection buffer is shipped frozen, but can be stored at 2-8°C in your premises.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below .

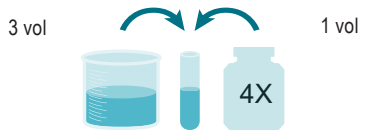
REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect 1X lysis buffer may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Lysis buffer and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- dsRNA standards (for standard curve) must be prepared in 1X lysis buffer or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

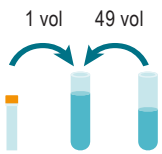
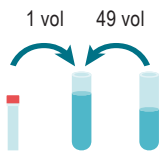
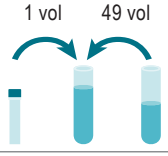
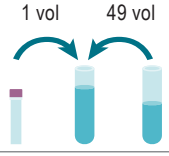
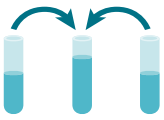
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 64RNAPEG		10,000 TESTS KIT - 64RNAPEH	
Anti-dsRNA Eu Cryptate antibody			
Thaw the dsRNA Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below .To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.			Thaw the dsRNA Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below .To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
Anti-dsRNA d2 antibody			
Thaw the dsRNA d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below .To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.			Thaw the dsRNA d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below .To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
dsRNA Standard			
To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.			To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.
Lysis buffer			
Prepare only the amount of lysis buffer needed for the experiment. Dilute 4-fold the 4 X lysis buffer#4 with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 0.5 mL of lysis buffer#4 + 1.5 mL of distilled water). Mix gently after dilution.			Prepare only the amount of lysis buffer needed for the experiment. Dilute 4-fold the 4 X lysis buffer#4 with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 0.5 mL of lysis buffer#4 + 1.5 mL of distilled water). Mix gently after dilution.
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL of dsRNA-Eu Cryptate Antibody and 5 µL of dsRNA-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 64RNAPEG		10,000 TESTS KIT - 64RNAPEH	
dsRNA Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA Eu Cryptate antibody with the detection buffer 1X: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer 1X (e.g., 5 µL of thawed Eu Cryptate antibody stock solution + 245 µL of Detection Buffer 1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA Eu Cryptate antibody with the detection buffer 1X: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of detection buffer 1X (e.g., 20 µL of thawed Eu Cryptate antibody stock solution + 980 µL of Detection Buffer 1X).
dsRNA d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA d2 antibody with the detection buffer 1X: add 1 volume of Cryptate antibody stock solution in 49 volumes of detection buffer 1X (e.g., 5 µL of thawed Cryptate-antibody stock solution + 245 µL of Detection Buffer 1X).			Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA d2 antibody with the detection buffer 1X: add 1 volume of Cryptate antibody stock solution in 49 volumes of detection buffer 1X (e.g., 20 µL of thawed Cryptate antibody stock solution + 980 µL of Detection Buffer 1X).
Antibody mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 250 µL of d2 antibody + 250 µL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with 1X lysis buffer #4
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in 1X lysis buffer #4 .
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

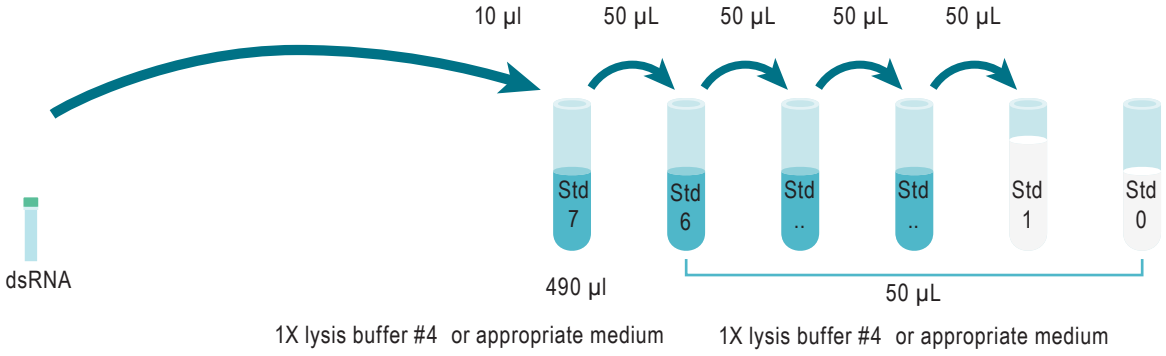
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 50-fold with 1X lysis buffer #4 to prepare high standard (Std 7): e.g. take 10 µl of standard stock solution and add it to 490 µl of 1X lysis buffer #4 . Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 50 µL of 1X lysis buffer #4 in each vial from Std 6 to Std 0.
- Add 50 µL of standard to 50 µL of 1X lysis buffer #4 , mix gently and repeat the 1/2 serial dilution to make standard solutions: std 6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is 1X lysis buffer #4 or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	DSRNA WORKING SOLUTIONS (ng/ml)
Standard Stock solution	Thawed stock solution	5,000
Standard 7	10µL Standard stock solution + 490 µL Lysis buffer 1X	100
Standard 6	50 µL Standard 7 + 50 µL Lysis buffer 1X	50
Standard 5	50 µL standard 6 + 50 µL lysis buffer 1X	25.0
Standard 4	50 µL standard 5 + 50 µL Lysis buffer 1X	12.50
Standard 3	50 µL standard 4 + 50 µL lysis buffer 1X	6.25
Standard 2	50 µL standard 3 + 50 µL Lysis buffer 1X	3.13
Standard 1	50 µL standard 2 + 50 µL Lysis buffer 1X	1.56
Standard 0	50 µL lysis buffer 1X	0

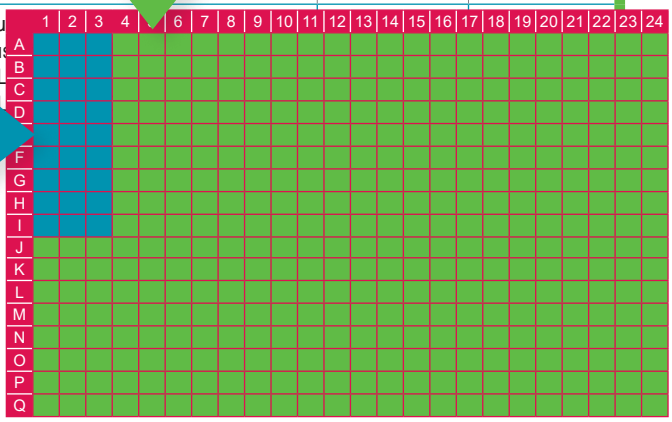
TO PREPARE SAMPLES:

- Each well requires 10 µL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- The Lysis Buffer should be used to prepare cell lysates after virus infection by the molecule of interest. After virus infection and incubation in 96 or 24-well plate, remove cell supernatant and add ice-cold 50-200 µL per well of 1X Lysis Buffer (depending on microplate size). Prepare cell lysate with pipetting or scraping and then transfer to the empty tube. Centrifuge and recover supernatant (cell lysate).
- Samples with a concentration above the highest standard (Std 7) must be diluted 1X lysis buffer #4

ASSAY PROTOCOL

	Standard (Std 0 - Std 7)	Samples
Step 1 	Dispense 10 µL of each dsRNA standard (Std 0 - Std 7) into each standard well	For the basal: dispense 10 µL of cells without viral infection into each sample well For the total amount: dispense 10 µL of cells with viral infection into each sample well
Step 2 	Add 5 µL of dsRNA d2 antibody working solution to all wells	
Step 3 	Add 5 µL of dsRNA Eu Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate overnight @ 4°C	
Step 5 	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	10 µL Std 0 (Negative control) 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well A1	Repeat Well A1	10 µL Sample 1 Non-virus infected cell lysate for the basal 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	10 µL Std 1 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well B1	Repeat Well B1	10 µL Sample 2 Virus infected cell lysate 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	10 µL Std 2 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well C1	Repeat Well C1	10 µL Sample 3 Non-virus infected cell lysate for the basal 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	10 µL Std ... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well D1	Repeat Well D1	10 µL Sample ... Virus infected cell lysate 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	10 µLStd ... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well E1	Repeat Well E1	10 µL Sample ... Non-virus infected cell lysate for the basal 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	10 µL Std ... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well F1	Repeat Well F1	10 µL Sample ... Virus infected cell lysate 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	10 µL Std ... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well G1	Repeat Well G1	10 µL Sample ... Non-virus infected cell lysate for the basal 5 µL dsRNA-d2 5 µL dsRNA-Eu C	Repeat Well G4	Repeat Well G4
H	10 µL Std ... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well H1	Repeat Well H1	10 µ Virus 5 µL		



DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

3. Calculate the delta ratio of the acceptor and donor emission signals for each individual well. The Standard 0 (Negative control) plays the role of an internal assay control.

delta Ratio = Ratio Standard or sample - Ratio Standard 0

For more information about data reduction, please visit http://www.cisbio.com/htrf-ratio-and-data-reduction

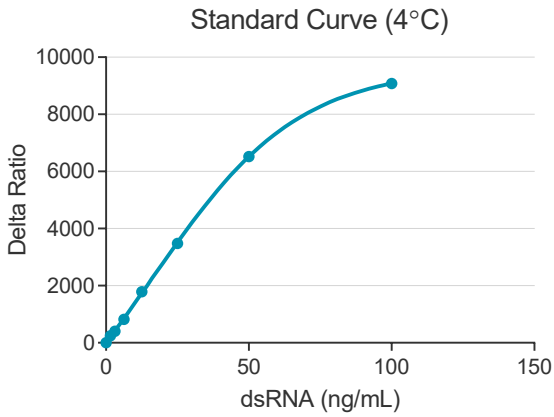
RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

Standard curve fitting with the 4 Parameter Logistic (4PL 1/Y²) model:

	Ratio (1)	CV (2)	Delta Ratio
Standard 0 - Negative control	574	3%	0
Standard 1 - 1.56 ng/mL	806	4%	232
Standard 2 - 3.13 ng/mL	968	8%	394
Standard 3 - 6.25 ng/mL	1378	7%	804
Standard 4 - 12.5 ng/mL	2349	1%	1775
Standard 5 - 25 ng/mL	4041	1%	3467
Standard 6 - 50 ng/mL	7084	1%	6510
Standard 7 - 100 ng/mL	9640	8%	9066



FOR MORE INFORMATION
Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors

PerkinElmer® | cisbio