MANUAL

Technology: HTRF™ Biomarkers

HTRF dsRNA (IVT) Kit

Part number:	64DSRNAPEG	64DSRNAPEH
Test size	500 tests	10,000 tests

Storage: ≤ -16°C or below

Version: 1 **Date:** July 2024

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of double-stranded RNA (dsRNA) particles in samples resulting from In Vitro Transcription (IVT) of mRNA. It offers a fast alternative to ELISA or immunoblot.

In Vitro Transcription is the process by which RNA transcripts are generated from a DNA template in Vitro, for research or therapeutic purposes. The desired outcome of IVT is a mix of single strand RNA (ssRNA) matching the DNA template, however, a common contaminant of this process are undesired dsRNA particles that can critically impact downstream applications, particularly therapeutic ones. dsRNA is normally not found in healthy animal cells, which prompted the innate immune system to use it as a way to detect viral infection or other issues. As a result, dsRNA contaminant in IVT samples are problematic because they can be immunogenic and trigger dangerous immune and inflammatory responses when injected to patients. To counter this, usual IVT processes include several steps of purification which lower dsRNA content and require reliable, easy-to-implement dsRNA detection techniques.

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 labelled with Europium cryptate (donor), and Anti-dsRNA 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 fluorescence at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the dsRNA concentration.

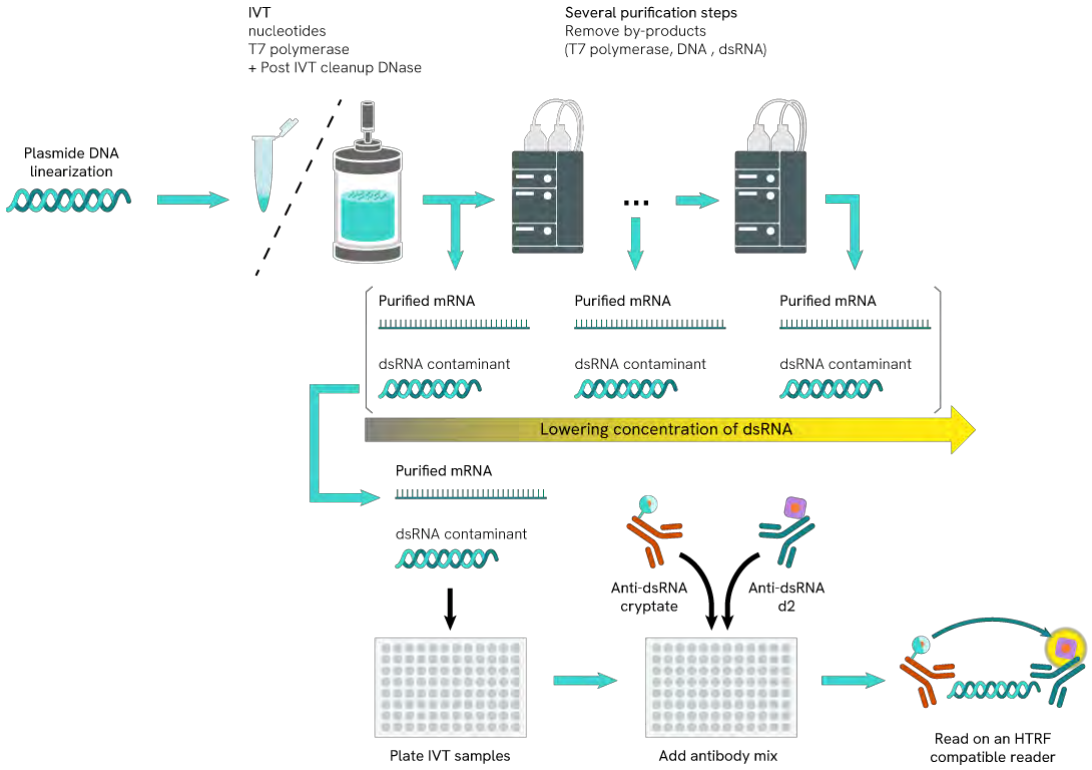
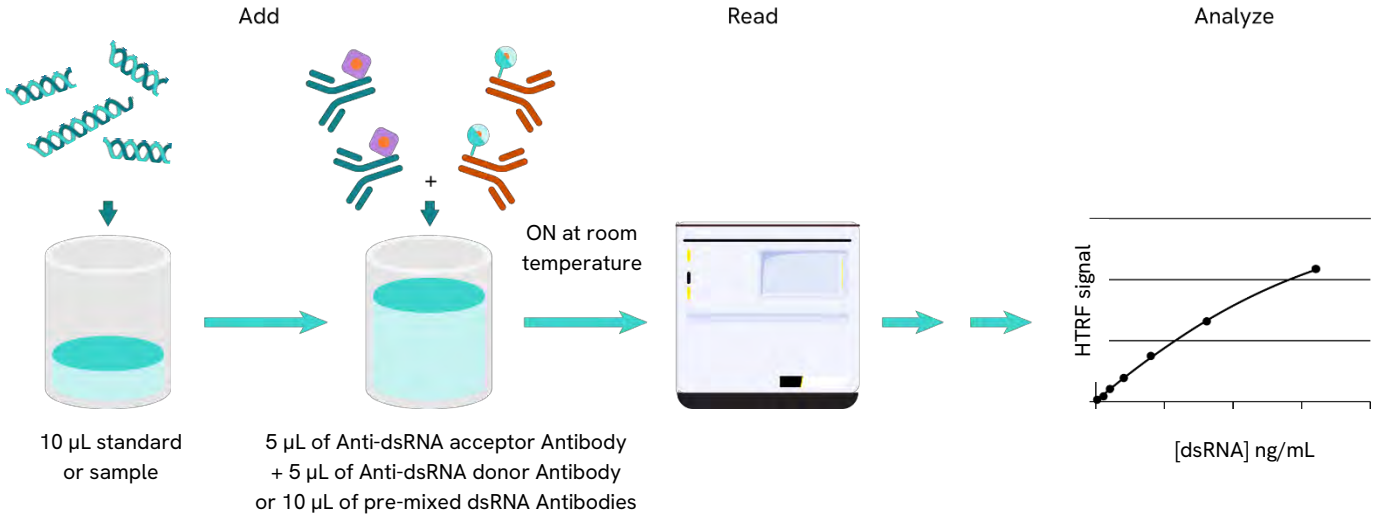


Figure 1: Principle of HTRF dsRNA (IVT) sandwich assay

PROTOCOL AT A GLANCE



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

MATERIAL PROVIDED

KIT COMPONENTS	500 TESTS*			10,000 TESTS*		
HTRF dsRNA (IVT) Kit - Std Frozen**		green cap	1 vial 1500 ng/mL		green cap	1 vial 1500 ng/mL
Anti-dsRNA Eu Cryptate Antibody Frozen 2X		orange cap	1 vial 1.25 mL		red cap	1 vial 25 mL
Anti-dsRNA d2 Antibody Frozen 50X		blue cap	1 vial 50 µL		purple cap	1 vial 1 mL
Diluent* #14 4X**		yellow cap	4 vials 2 mL		white cap	1 vial 130 mL
Detection Buffer** #19 Ready-to-use**		transparent cap	1 vial 7 mL		red cap	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.
** Catalog Synthetic dsRNA Control also available separately Cat#64RNATDA.
*** Dilute with RNase free water.
**** 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 our website.
- Small volume (SV) detection microplates. Use white plate only. For more information about microplate recommendations, please visit our website.

STORAGE AND STABILITY

Kit

- Store the kit at -16°C or below.
- Under proper storage conditions, reagents are stable until the expiry date indicated on the label.
- Diluent and detection buffer are shipped frozen but can be stored at 2-8°C in your premises.

Reagents

- Once thawed, antibody and standard solutions can be frozen once.
- To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below for Standard and for antibody solutions.
- Volume of standard and antibody aliquots should not be under 10 µL.
- Thawed diluent can be stored at 2-8°C in your premises for 1 week. For long term storage, it is recommended to store the diluent frozen at -16°C or below.
- Thawed detection buffer can be stored at 2-8°C in your premises.

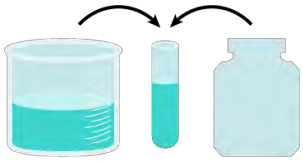
REAGENT PREPARATION

Before you begin

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent 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 45 mins before use.
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- It is recommended to filter buffers.
- Antibody solutions and premix must be prepared in individual vials.
- dsRNA standards (for standard curve) must be prepared in diluent.

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

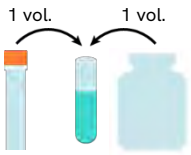
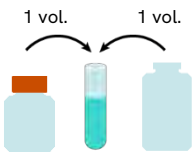
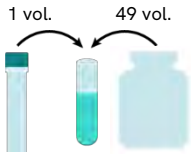
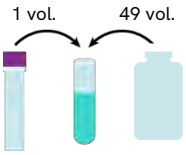
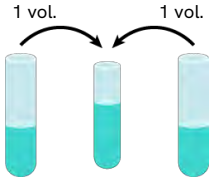
To prepare reagent stock solutions

500 TESTS		10,000 TESTS	
Anti-dsRNA Eu Cryptate Antibody			
Thaw the dsRNA Eu Cryptate Antibody. Mix gently. This 2X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.			Thaw the dsRNA Eu Cryptate Antibody. Mix gently. This 2X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
Anti-dsRNA d2 Antibody			
Thaw the dsRNA d2 Antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.			Thaw the dsRNA d2 Antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
dsRNA Standard			
Thaw the dsRNA standard to obtain 1500 ng/mL stock solution. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.			Thaw the dsRNA standard to obtain 1500 ng/mL stock solution. Mix gently. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.
Diluent			
Dilute 4-fold the 4 X diluent #14 with RNase free water: homogenize the 4 X diluent #14 with a vortex and add 1 volume of stock solution in 3 volumes of RNase free water (e.g., 1 mL of diluent + 3 mL of water). Mix gently after dilution.			Dilute 4-fold the 4 X diluent #14 with RNase free water: homogenize the 4 X diluent #14 with a vortex and add 1 volume of stock solution in 3 volumes of RNase free water (e.g., 20 mL of diluent + 60 mL of water). Mix gently after dilution.
Detection buffer			
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.

<table>
<tr><th colspan="2">500 TESTS</th><th colspan="2">10,000 TESTS</th></tr>
<tr><td colspan="4">dsRNA Eu Cryptate Antibody</td></tr>
<tr><td>Dilute 2-fold the 2X stock solution (thawed reagent) of dsRNA Eu Cryptate Antibody with Detection buffer #19: add 1 volume of Eu Cryptate Antibody stock solution in 1 volume of detection buffer (e.g. 1.25 mL of Eu Cryptate Antibody stock solution + 1.25 mL of detection buffer).</td><td> 
</td><td> 
</td><td>Dilute 2-fold the 2X stock solution (thawed reagent of dsRNA Eu Cryptate Antibody with Detection buffer #19: add 1 volume of Eu Cryptate Antibody stock solution in 1 volume of detection buffer (e.g. 25 mL of Eu Cryptate Antibody stock solution + 25 mL of detection buffer).</td></tr>
<tr><td colspan="4">dsRNA d2 antibody</td></tr>
<tr><td>Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA d2 Antibody with Detection buffer #19: add 1 volume of d2 antibody stock solution in 49 volumes of detection buffer (e.g. 50 µL of d2 Antibody stock solution + 2450 µL of detection buffer).</td><td> 
</td><td> 
</td><td>Dilute 50-fold the 50X stock solution (thawed reagent) of dsRNA d2 Antibody with Detection buffer #19: add 1 volume of d2 Antibody stock solution in 49 volumes of detection buffer (e.g. 1 mL of d2 Antibody stock solution + 49 mL of detection buffer).</td></tr>
<tr><td colspan="4">Antibody Mix</td></tr>
<tr><td>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. 2.5 mL of d2 Antibody + 2.5 mL of Cryptate Antibody).</td><td colspan="2"> 
</td><td>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. 50 mL of d2 Antibody + 50 mL of Cryptate Antibody).</td></tr>
</table>

To prepare working standards solutions

- Each well requires 10 µL of standard.
- Dilute the standard stock solution serially with diluent #14 (1X).
- 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 diluent #14 (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

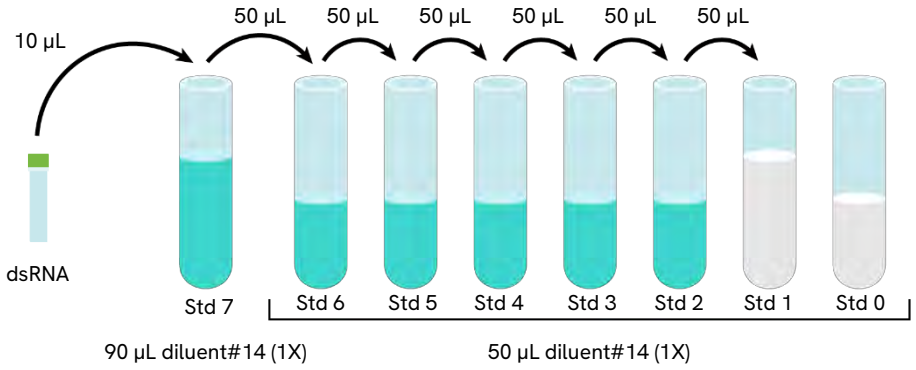
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 10-fold with diluent #14 (1X) to prepare high standard (Std 7): e.g. take 10 µL of standard stock solution and add it to 90 µL of diluent #14 (1X). Mix gently.

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

- Dispense 50 µL of diluent #14 (1X) in each vial from Std 6 to Std 0.
- Add 50 µL of standard to 50 µL of diluent #14 (1X), mix gently and repeat the 1/2 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is diluent #14 (1X) alone



STANDARD	SERIAL DILUTIONS	WORKING SOLUTIONS
Standard Stock solution	Thawed stock solution	1 500 ng/mL
Standard 7	10 µL Standard stock Solution + 90 µL diluent 1X	150 ng/mL
Standard 6	50 µL standard 7 + 50 µL diluent 1X	75 ng/mL
Standard 5	50 µL standard 6 + 50 µL diluent 1X	37.5 ng/mL
Standard 4	50 µL standard 5 + 50 µL diluent 1X	18.75 ng/mL
Standard 3	50 µL standard 4 + 50 µL diluent 1X	9.375 ng/mL
Standard 2	50 µL standard 3 + 50 µL diluent 1X	4.69 ng/mL
Standard 1	50 µL standard 2 + 50 µL diluent 1X	2.34 ng/mL
Standard 0	50 µL diluent 1X	0

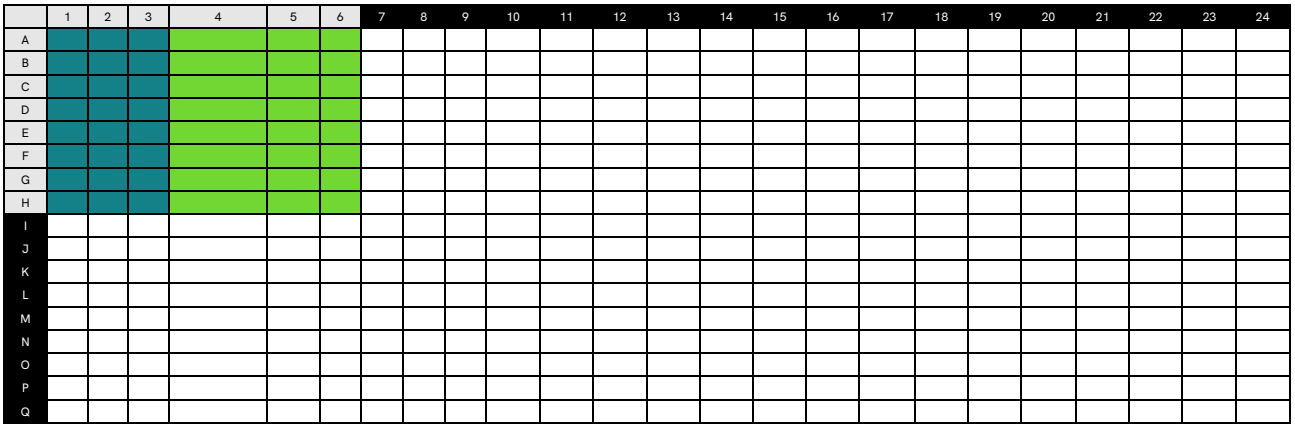
To prepare samples

- 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 -16°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted in diluent #14 (1X).
- Ionic strength tolerance: The kit tolerates undiluted samples detection without impairing assay performance until 0.5M of ionic strength. In case of high ionic strength condition, sample dilution is required (at least 10X for 0.5M and 40X for 1M).

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	Dispense 10 µL of each sample 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 at RT Following incubation, the signal remains stable over a period of 48 hours.	
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 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 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 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	10 µL Std 3 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well D1	Repeat Well D1	10 µL sample... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	10 µL Std 4 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well E1	Repeat Well E1	10 µL sample... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	10 µL Std 5 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well F1	Repeat Well F1	10 µL sample... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	10 µL Std 6 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well G1	Repeat Well G1	10 µL sample... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well G4	Repeat Well G4
H	10 µL Std 7 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well H1	Repeat Well H1	10 µL sample... 5 µL dsRNA-d2 5 µL dsRNA-Eu Cryptate	Repeat Well H4	Repeat Well H4



DATA REDUCTION & INTERPRETATION

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 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.

$$\text{delta Ratio} = \text{Ratio Standard or sample} - \text{Ratio Standard 0}$$

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

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

For more information about data reduction, please visit our website.

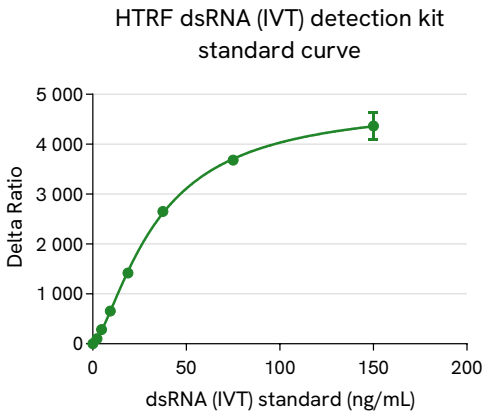
RESULTS

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 with $1/Y^2$) model

		Ratio (1)	Delta Ratio (2)	CV% (3)
Std 0	**Negative control**	223	0	0%
Std 1	2.34 ng /mL	325	102	1%
Std 2	4.69 ng /mL	508	285	1%
Std 3	9.37 ng /mL	876	655	3%
Std 4	18.75 ng /mL	1642	1419	4%
Std 5	37.5 ng /mL	2876	2653	3%
Std 6	75 ng /mL	3906	3681	2%
Std 7	150 ng /mL	4590	4366	6%



REACH European regulations and compliance.

REACH European regulations and compliance. 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.

Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

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