MANUAL

HTRF HUMAN/MOUSE TOTAL HUNTINGTIN (HTT) DETECTION KITS

Part # 64HTTTPEG & 64HTTTPEH

Test Size#: 500 TESTS (64HTTTPEG), 10,000 TESTS (64HTTTPEH)

Revision: #03 of February 2024 **Store at:** ≤-60°C

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of soluble total HTT (Huntingtin) protein in cell/tissue lysates. HTT is produced by cells and after lysis of the cell membrane, the level of soluble total HTT (wild-type and mutant forms) can be detected using the kit reagents.

As shown in Figure 1, Total HTT is detected in a sandwich assay format using 2 different specific monoclonal antibodies, one labeled with Tb^{3+} Cryptate (donor), and the second labeled with d2 (acceptor).

The detection principle is based on HTRF® technology. 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). The two antibodies bind to the Total HTT protein present in the sample, thereby generating FRET. Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Total HTT protein concentration (Fig. 1).

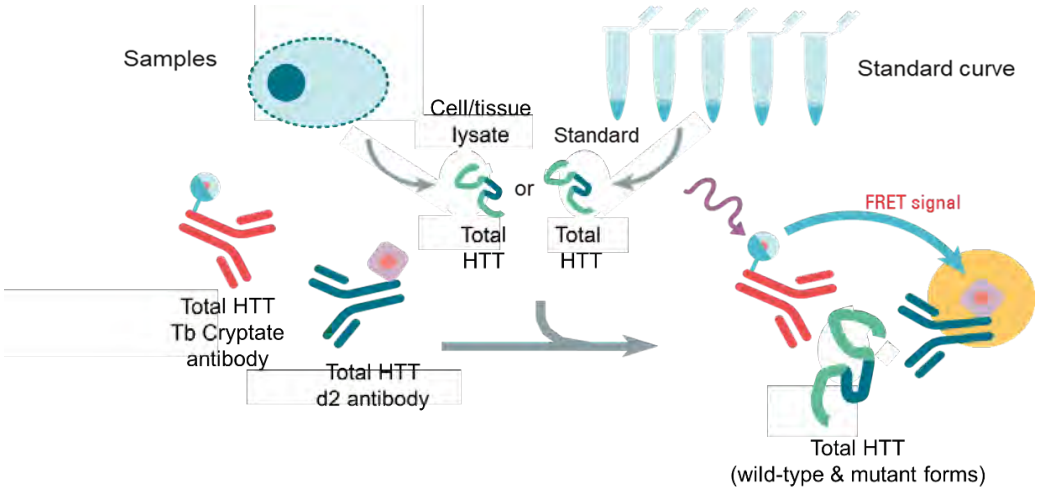
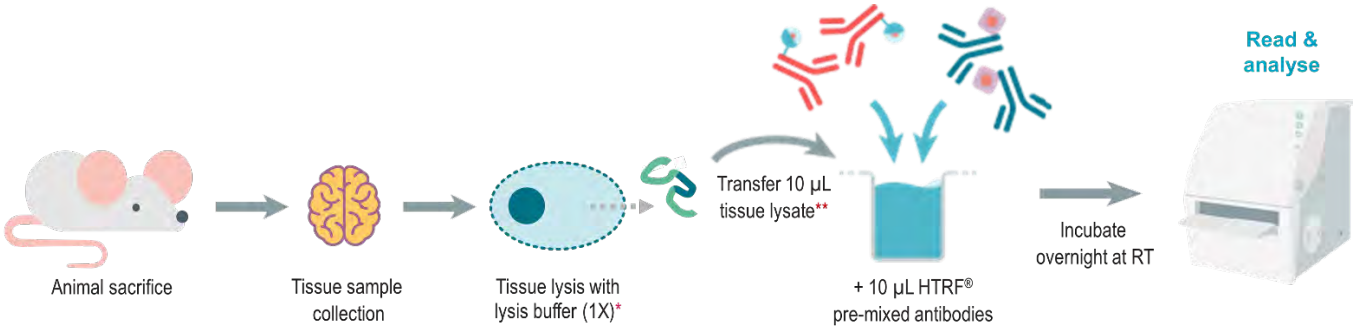


Figure 1: Principle of HTRF Total HTT sandwich assay

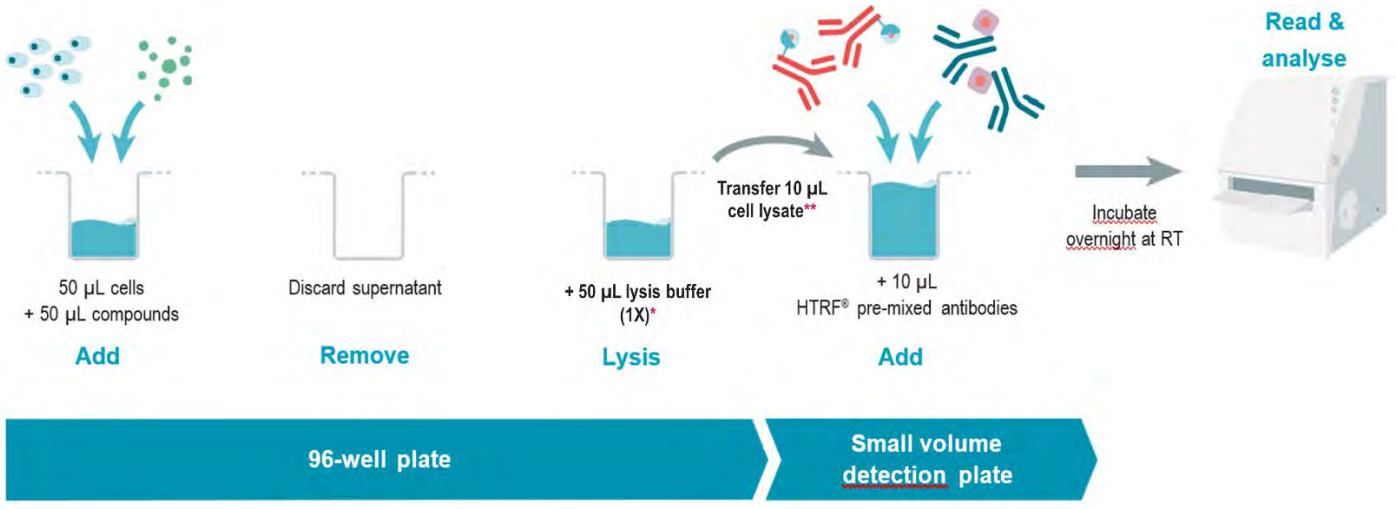
MANUAL AT A GLANCE

► ASSAY MANUAL FOR TISSUES:

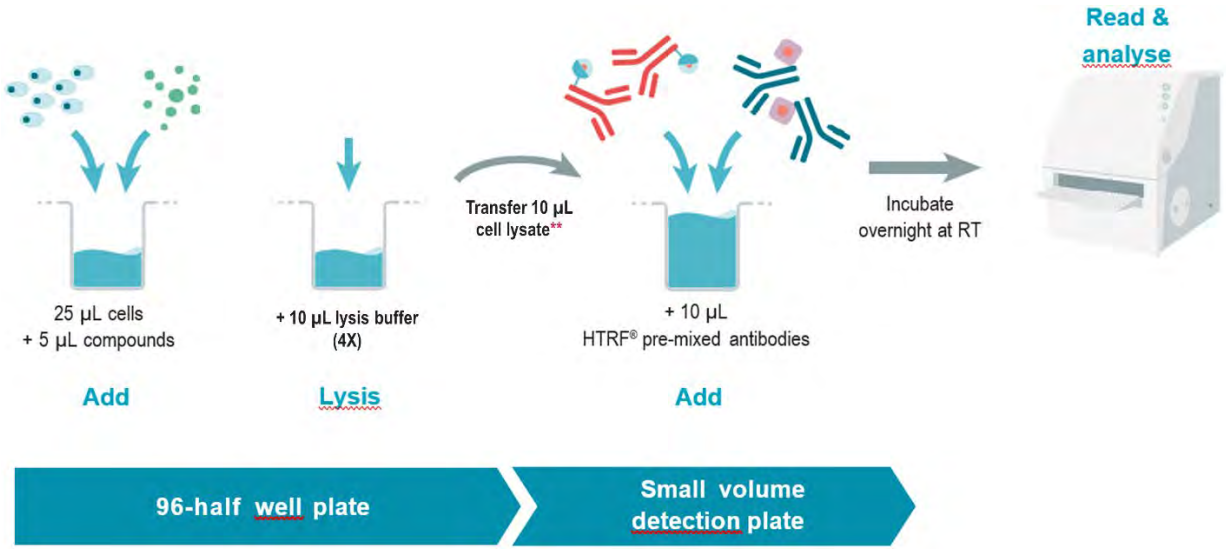


* The lysis buffer must be supplemented with protease inhibitors. For more details about tissue lysate preparation, please refer to the Technical Note "Optimize Your HTRF® Cell Signaling Assays on Tissues" on www.revvity.com. Depending on tissue samples, volume of lysis should be optimized. ** Before detection, it can be necessary to dilute the tissue lysate in 1X lysis buffer supplemented with protease inhibitors to ensure that the detected analyte will be assessed at a concentration compatible with the assay's linear range.

► TWO- PLATE ASSAY MANUAL FOR ADHERENT CELLS:



► TWO- PLATE ASSAY MANUAL FOR SUSPENSION CELLS:



* Depending on cell lines used, volume of lysis should be optimized.
** Before detection, it can be necessary to dilute the cell lysate in 1X lysis buffer to ensure that the detected analyte will be assessed at a concentration compatible with the assay's linear range.

MATERIALS PROVIDED:

KIT COMPONENTS	STORAGE	500 TESTS* CAT# 64HTTTPEG		10,000 TESTS* CAT# 64HTTTPEH	
Total HTT Tb cryptate antibody (stock solution 50X)	≤-16°C	Orange cap	1 vial - 50 µL	Red cap	1 vial – 1 mL
Total HTT d2 antibody (stock solution 50X)	≤-16°C	Blue cap	1 vial - 50 µL	Purple cap	1 vial – 1 mL
Total HTT control lysate (ready-to-use)	≤-60°C	 Green cap	1 vial - 150 µL	 Green cap	2 vials - 150 µL
Lysis buffer** # 2 (stock solution 4X)	≤-16°C	Transparent cap	4 vials – 2 mL	White cap	1 vial – 130 mL
Detection buffer*** # 15 (ready-to-use)	≤-16°C	Orange cap	3 vials – 2 mL	 Red cap	2 vials – 50 mL

*When used as advised, the two available kit sizes will provide sufficient reagents for 500 and 10,000 tests respectively in 20 µL final. Assay volumes can be adjusted proportionally to run the assay in 96-well or 1536-well microplates.

** The amount of lysis buffer provided is sufficient for generating 50 µL of cell lysate per well.

*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

► PURCHASE SEPARATELY

*HTRF®-Certified Reader. Make sure the setup for Tb Cryptate is used.
For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

*Small volume (SV) detection microplates.
For more information about microplate recommendations, please visit our website at: www.revvity.com

*The following recombinant wild-type (WT) and mutant HTT proteins can be optionally used for absolute quantification: WT mouse HTT-Q7 (Coriell Institute, #CH03222), WT human HTT-Q23 (Coriell Institute, #CH03333), mutant human HTT-Q48 (Coriell Institute, #CH03188) and mutant human HTT-Q73 (Coriell Institute, #CH03267).

STORAGE AND STABILITY

Storage upon reception:

Store the kit at -60°C or below until the expiration date indicated on the package.

Storage and stability of thawed material:

When you are ready to use the kit, take the reagents out and prepare them following the manual provided in this document. Unused thawed reagents can be stored and conserved for future use. Refer to the table below for storage options and corresponding shelf life.

	Storage after thawing
Lysis Buffer / Detection buffer	2-8°C until the expiration date indicated on the package
Antibodies*	2-8°C for 48h or freeze at -16°C or below until the expiration date indicated on the package for long term storage
Control Lysate*	Freeze at -60°C or below until the expiration date indicated on the package for long term storage

*Stock solutions may be thawed and frozen only once. Freeze in aliquots to avoid multiple freeze/thaw cycles (once aliquoted, single use of the reagent). Volume of antibodies aliquots should not be under 10 µL. Volume of control lysate aliquots should not be under 20µL.

REAGENT PREPARATION

It is very important to prepare cell/tissue lysates and detection reagents in the kit buffers. The use of an incorrect lysis buffer or diluent may affect assay results and reagent stability.

Allow all reagents to thaw before use. We recommend centrifuging the vials gently after thawing, before pipetting the stock solutions.

Prepare the working solutions from stock solutions by following the instructions below.

TO PREPARE WORKING CONTROL LYSATE SOLUTION:

The control lysate is only provided as an internal assay control to check the quality of the results obtained. The window between control lysate and negative control should be greater than 2.

Thaw the control lysate. Mix gently, the control lysate is ready-to-use.

TO PREPARE WORKING ANTIBODY SOLUTIONS:

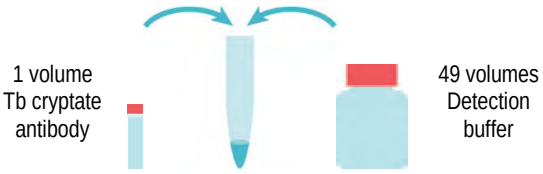
HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 or

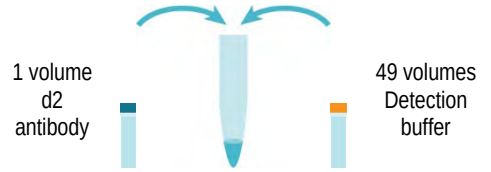
Terbium Cryptate-antibodies will impair the assay's quality.

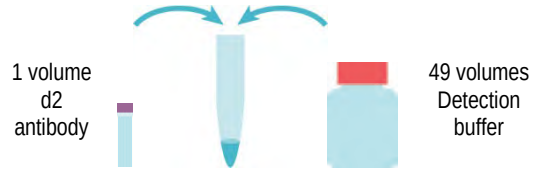
Each well requires 5 µL of Total HTT Tb cryptate antibody and 5 µL of Total HTT d2 antibody. Prepare the two antibody solutions in separate vials. Antibody working solutions are stable for 2 days at 2-8°C.

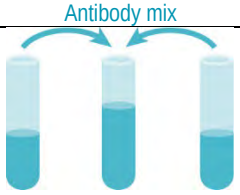
Dilute the antibodies with detection buffer. In practice:

<table>
<tr><th>500 TESTS KIT CAT# 64HTTTPEG</th><th>10,000 TESTS KIT CAT# 64HTTTPEH</th></tr>
<tr><td colspan="2">Total HTT Tb cryptate antibody</td></tr>
<tr><td> 

Dilute the frozen stock solution with detection buffer 50-fold, e.g., add 2.45 mL of detection buffer to 0.05 mL of Tb cryptate-antibody stock solution.</td><td> 

Dilute the frozen stock solution with detection buffer 50-fold, e.g., add 49 mL of detection buffer to 1 mL of Tb cryptate-antibody stock solution.</td></tr>
<tr><td colspan="2">Total HTT d2 antibody</td></tr>
<tr><td> 

Dilute the frozen stock solution with detection buffer 50-fold, e.g., add 2.45 mL of detection buffer to 0.05 mL of d2-antibody stock solution.</td><td> 

Dilute the frozen stock solution with detection buffer 50-fold, e.g., add 49 mL of detection buffer to 1 mL of d2-antibody stock solution.</td></tr>
<tr><td colspan="2">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 Tb Cryptate-antibody solution.</td><td>  </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 Tb cryptate-antibody solution.</td></tr>
</table>

TO PREPARE LYSIS BUFFER USED FOR CELL/TISSUE LYSIS AND STANDARD DILUTION:

Make sure that the cell/tissue lysates have been generated by using the kit lysis buffer.
The lysis buffer differs between the manuals. Make sure you use the appropriate lysis buffer depending on the chosen manual's specifications.

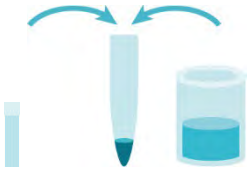
Prepare the required amount of lysis buffer before running the assay. Working solutions are stable for 2 days at 2-8°C.

► ***Lysis buffer 1X for assay manual on tissues, two-plate assay manual on adherent cells, and standard dilution***

Determine the amount of lysis buffer needed for the experiment.

The two-plate assay manual on adherent cells generally requires 50 µL of 1X lysis buffer/well.

Prepare a lysis buffer solution 1X and use it to lyse adherent cells or tissues, or to prepare standard dilutions. In practice:

<table>
<tr><th colspan="2">ASSAY MANUAL ON TISSUES, TWO-PLATE ASSAY MANUAL ON ADHERENT CELLS, STANDARD DILUTION</th></tr>
<tr><th colspan="2">500 TESTS KIT CAT# 64HTTTPEG & 10,000 TESTS KIT CAT# 64HTTTPEH</th></tr>
<tr><td colspan="2">Preparation of lysis buffer 1X</td></tr>
<tr><td>500 TESTS</td><td>10,000 TESTS</td></tr>
<tr><td>1 volume
Lysis buffer
4X
 
3 volumes
Distilled
water
.</td><td>1 volume
Lysis buffer
4X
 
3 volumes
Distilled
water</td></tr>
<tr><td colspan="2">Dilute the “lysis buffer 4X” 4-fold with distilled water to prepare lysis buffer 1X.
e.g., take 2 mL of lysis buffer 4X and add it to 6 mL of distilled water. Mix gently.</td></tr>
</table>

► ***Lysis buffer 4X for two-plate assay manual on suspension cells***

Determine the amount of lysis buffer needed for the experiment. Each well requires 10 µL of 4X lysis buffer/well.

The lysis buffer 4X is ready-to-use.

TO PREPARE TISSUE SAMPLES:

Please refer to the Technical Note “Optimize Your HTRF® Cell Signaling Assays on Tissues” on www.revvity.com.

It is recommended to prepare a 10% or 5% (w/v) tissue homogenate (e.g., 100 mg or 50 mg of tissue lyzed with 1 mL of lysis buffer) using ice-cold 1X lysis buffer #2 supplemented with protease inhibitors. Depending on tissue samples, volume of lysis should be optimized.

After tissue lysis, insoluble fractions containing putative mutant HTT aggregates must be removed by centrifugation at 3500xg for 10 minutes at 4°C, and the supernatants containing soluble total HTT are collected to be directly analysed or dispensed into aliquots for storage at ≤-60°C. Frozen supernatants must be rapidly assayed within a few days as HTT tends to aggregate in lysates over time.

Just before detection, it can be necessary to dilute tissue samples in 1X lysis buffer supplemented with protease inhibitors to ensure that the detected analyte will be assessed at a concentration compatible with the assay’s linear range.

TO PREPARE STANDARD WORKING SOLUTIONS (OPTIONAL):

No standard is provided in the kit. The following recombinant WT and mutant HTT proteins can be purchased separately for absolute quantification of total HTT in cell/tissue lysates: WT mouse HTT-Q7 (Coriell Institute, #CH03222), WT human HTT-Q23 (Coriell Institute, #CH03333), mutant human HTT-Q48 (Coriell Institute, #CH03188) and mutant human HTT-Q73 (Coriell Institute, #CH03267).

• Each well requires 10 µL of standard.

• Dilute the standard stock solution serially with lysis buffer #2 (1X).

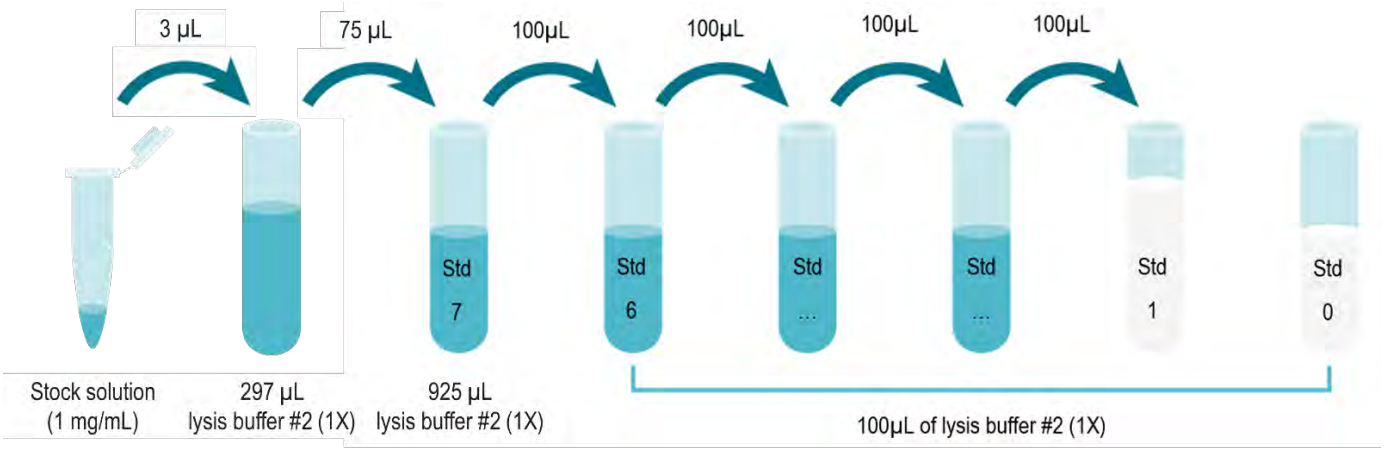
• In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

An intermediary 1/100 dilution of the standard stock solution (at 1 mg/mL) is first done to prepare a solution at 10,000 ng/mL. The high standard (Std 7) is then prepared by doing a 1/13.3 dilution of the intermediary solution. Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

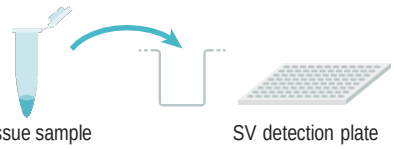
• Dispense 100 µL of lysis buffer #2 (1X) in each vial from Std 6 to Std 0.

- Add 100 µL of standard (Std 7) to 100 µL of lysis buffer #2 (1X), mix gently and repeat the 1/2 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.
- Std 0 is lysis buffer #2 (1X) only.



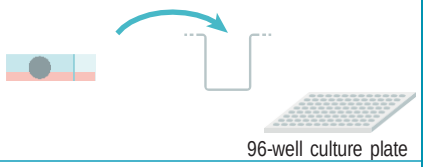
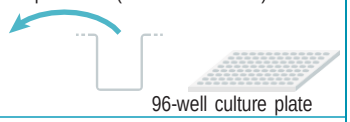
STANDARD	SERIAL DILUTIONS	TOTAL HTT STANDARD WORKING SOLUTIONS (ng/mL)
Intermediary dilution	3 µL stock solution (at 1 mg/mL) + 297 µL lysis buffer #2 (1X)	10,000
Standard 7	75 µL intermediary dilution + 925 µL lysis buffer #2 (1X)	750
Standard 6	100 µL standard 7 + 100 µL lysis buffer #2 (1X)	375
Standard 5	100 µL standard 6 + 100 µL lysis buffer #2 (1X)	187.5
Standard 4	100 µL standard 5 + 100 µL lysis buffer #2 (1X)	93.8
Standard 3	100 µL standard 4 + 100 µL lysis buffer #2 (1X)	46.9
Standard 2	100 µL standard 3 + 100 µL lysis buffer #2 (1X)	23.4
Standard 1	100 µL standard 2 + 100 µL lysis buffer #2 (1X)	11.7
Standard 0	100 µL lysis buffer #2 (1X)	0

ASSAY MANUAL FOR TISSUES

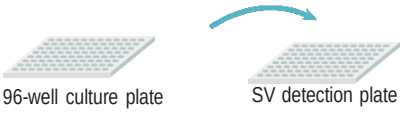
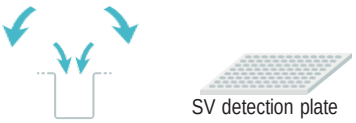
1	Transfer 10 µL of tissue lysate to a small volume (SV) white detection plate. Depending on the type of tissue used, it can be necessary to dilute the lysate to ensure samples are within the assay linear range	
2	Add 10 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer. Cover the plate with a plate sealer. Incubate overnight at room temperature. Set up your reader for Tb^{3+} Cryptate and read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on a compatible HTRF® reader.	

TWO-PLATE ASSAY MANUAL FOR ADHERENT AND SUSPENSION CELLS

GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS PREPARATION

	FOR ADHERENT CELLS	FOR SUSPENSION CELLS	
1	Plate 50 µL of cells in 96-well tissue-culture treated plate in appropriate growth medium and incubate overnight, at 37°C in CO2 atmosphere.	Plate 25 µL of cells in 96 half-well plate in your appropriate medium.	 96-well culture plate
	Cell seeding densities of 100K cells/well are generally sufficient for most cell lines, but optimization of cell seeding densities is recommended.		
2	Dispense 50 µL of compound (2X) diluted in cell culture medium.	Dispense 5 µL of compound (6X), diluted in your appropriate medium.	 96-well culture plate
	For most compound, incubation time is between 30 minutes and 16 hours at 37°C. We recommend a time course study to determine the optimal time of treatment.		
3	Carefully remove cell supernatant either by aspirating supernatant or by flicking the plate.	Do not remove your appropriate medium.	Discard supernatant (for adherent cells)  96-well culture plate

TOTAL HTT DETECTION USING HTRF KIT

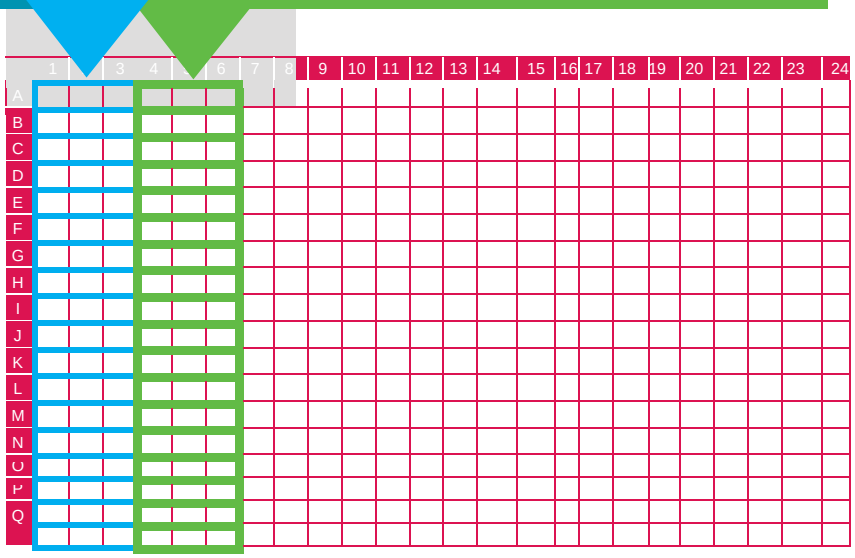
	FOR ADHERENT CELLS	FOR SUSPENSION CELLS	
4	Immediately add 50 µL of lysis buffer (1X) and incubate for at least 30 minutes at room temperature under shaking.	Immediately add 10 µL of lysis buffer (4X) and incubate for at least 30 minutes at room temperature under shaking.	 96-well culture plate
	Use the appropriate lysis buffer and incubate at room temperature with shaking. Lysis incubation time may be optimized. Lysis volume can be decreased down to 25 µL.		
5	After homogenization by pipeting up and down, transfer 10 µL of cell lysate from the 96-well cell-culture plate to a small volume (SV) white detection plate. Depending on cell lines used, it can be necessary to dilute the cell lysate to ensure samples are within the assay linear range		 96-well culture plate SV detection plate
6	Add 10 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer. Cover the plate with a plate sealer. Incubate overnight at room temperature. Set up your reader for Tb^{3+} Cryptate and read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on a compatible HTRF® reader.		 SV detection plate

STANDARD MANUAL FOR DETECTION IN 20 µL FINAL VOLUME

	CELL/TISSUE LYSATES	STANDARDS (optional)	CONTROL LYSATE	NEGATIVE CONTROL
Step 1	Dispense 10 µL of each cell/tissue lysate (triplicates)	Dispense 10 µL of each dilution of standard (triplicates)	Dispense 10 µL of control lysate (triplicate)	Dispense 10 µL of lysis buffer (1X) (triplicate)
Step 2	Add 5 µL of Total HTT d2 antibody working solution to all wells.			
Step 3	Add 5 µL of Total HTT Tb cryptate antibody working solution to all wells.			
Step 4	Cover the plate with a plate sealer. Incubate overnight at room temperature. 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.			

The Negative control is used to check the non-specific signal.

	1	2	3	4	5	6
A	10 µL Lysis Buffer #2 (1X) ***Negative control*** 10 µL pre-mixed Total HTT antibodies	Repeat Well A1	Repeat Well A1	10 µL Standard 0 ***Standard 0*** 10 µL pre-mixed Total HTT antibodies	Repeat Well A4	Repeat Well A4
B	10 µL Total HTT control lysate ***Control lysate*** 10 µL pre-mixed Total HTT antibodies	Repeat Well B1	Repeat Well B1	10 µL Standard 1 ***Standard 1*** 10 µL pre-mixed Total HTT antibodies	Repeat Well B4	Repeat Well B4
C	10 µL cell/tissue lysate 1 ***Sample 1*** 10 µL pre-mixed Total HTT antibodies	Repeat Well C1	Repeat Well C1	10 µL Standard 2 ***Standard 2*** 10 µL pre-mixed Total HTT antibodies	Repeat Well C4	Repeat Well C4
D	10 µL cell/tissue lysate 2 ***Sample 2*** 10 µL pre-mixed Total HTT antibodies	Repeat Well D1	Repeat Well D1	10 µL Standard 3 ***Standard 3*** 10 µL pre-mixed Total HTT antibodies	Repeat Well D4	Repeat Well D4
E	10 µL cell/tissue lysate 3 ***Sample 3*** 10 µL pre-mixed Total HTT antibodies	Repeat Well E1	Repeat Well E1	10 µL Standard 4 ***Standard 4*** 10 µL pre-mixed Total HTT antibodies	Repeat Well E4	Repeat Well E4
F	10 µL cell/tissue lysate … ***Sample …*** 10 µL pre-mixed Total HTT antibodies	Repeat Well F1	Repeat Well F1	10 µL Standard 5 ***Standard 5*** 10 µL pre-mixed Total HTT antibodies	Repeat Well F4	Repeat Well F4
G	10 µL cell/tissue lysate … ***Sample …*** 10 µL pre-mixed Total HTT antibodies	Repeat Well G1	Repeat Well G1	10 µL Standard 6 ***Standard 6*** 10 µL pre-mixed Total HTT antibodies	Repeat Well G4	Repeat Well G4
H	10 µL cell/tissue lysate … ***Sample …*** 10 µL pre-mixed Total HTT antibodies	Repeat Well H1	Repeat Well H1	10 µL Standard 7 ***Standard 7*** 10 µL pre-mixed Total HTT antibodies	Repeat Well H4	Repeat Well H4



DATA REDUCTION & INTERPRETATION

1. Calculate the Ratio of the acceptor and donor emission signals for each well:

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

2. Calculate the Delta Ratio for each well. The Negative control (or Standard 0) plays the role of an internal assay control.

$$\text{Delta Ratio} = \text{HTRF Ratio}_{\text{Sample (or Standard)}} - \text{HTRF Ratio}_{\text{Negative control (or Standard 0)}}$$

3. The Mean Delta Ratio and Standard Deviation can then be worked out from Delta Ratio replicates. Calculate the CV (%) for each replicate:

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

For more information about data reduction, please visit www.revvity.com

RESULTS

These data should be considered only as an example. Results may vary from one HTRF® compatible reader to another.

1. Results on mouse brain tissue samples, using the assay manual for tissues.

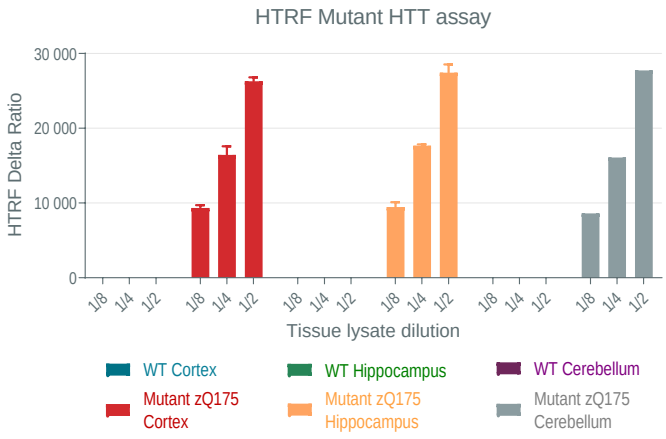
Several brain tissues (cortex, hippocampus, and cerebellum) were collected from a WT HTT mouse model and from a mutant zQ175 HTT mouse model. Tissue homogenates were prepared as described on page 5 in the section “To prepare tissue samples”. All samples were serially diluted in lysis buffer #2 (1X) supplemented with protease inhibitors.

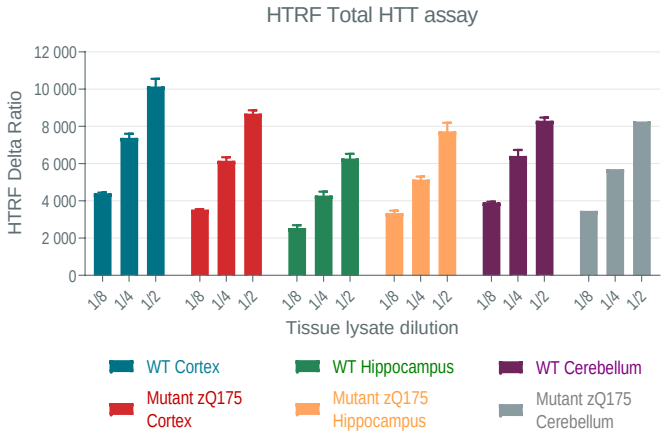
10 µL of lysates were transferred in a SV white detection plate to detect soluble Total HTT, and 10 µL of lysates were transferred in separate wells of the plate to detect soluble Mutant HTT using the HTRF Mutant HTT assay - Cat # 64HTTMPEG, 64HTTMPEH.

The histobars represent the HTRF Delta Ratio obtained for each experimental condition.

		Mutant HTT		Total HTT	
Mouse brain tissue	Lysate dilution	Mean Delta Ratio	CV%	Mean Delta Ratio	CV%
WT Cortex	1/8	0	-	4401	1%
	1/4	0	-	7383	3%
	1/2	0	-	10148	4%
Mutant zQ175 Cortex	1/8	9327	4%	3524	1%
	1/4	16445	7%	6158	3%
	1/2	26277	2%	8687	2%
WT Hippocampus	1/8	0	-	2541	6%
	1/4	0	-	4280	5%
	1/2	0	-	6276	4%
Mutant zQ175 Hippocampus	1/8	9454	7%	3344	4%
	1/4	17661	1%	5147	3%
	1/2	27429	4%	7734	6%

		Mutant HTT		Total HTT	
Mouse brain tissue	Lysate dilution	Mean Delta Ratio	CV%	Mean Delta Ratio	CV%
WT Cerebellum	1/8	0	-	3915	1%
	1/4	0	-	6412	5%
	1/2	0	-	8309	2%
Mutant zQ175 Cerebellum	1/8	8606	2%	3463	2%
	1/4	16093	7%	5705	2%
	1/2	27735	2%	8266	4%



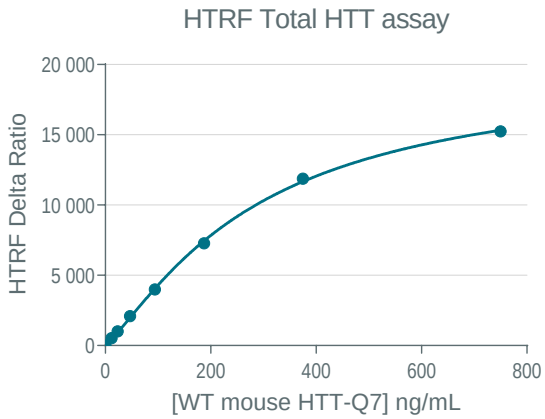


2. Example of standard curve obtained with the recombinant WT mouse HTT-Q7 protein (Coriell Institute, #CH02324).

The data are drawn up by plotting the HTRF Delta Ratio versus [Standard] (ng/mL).

Standard curve fitting with the 4 Parameter Logistic (4PL) model.

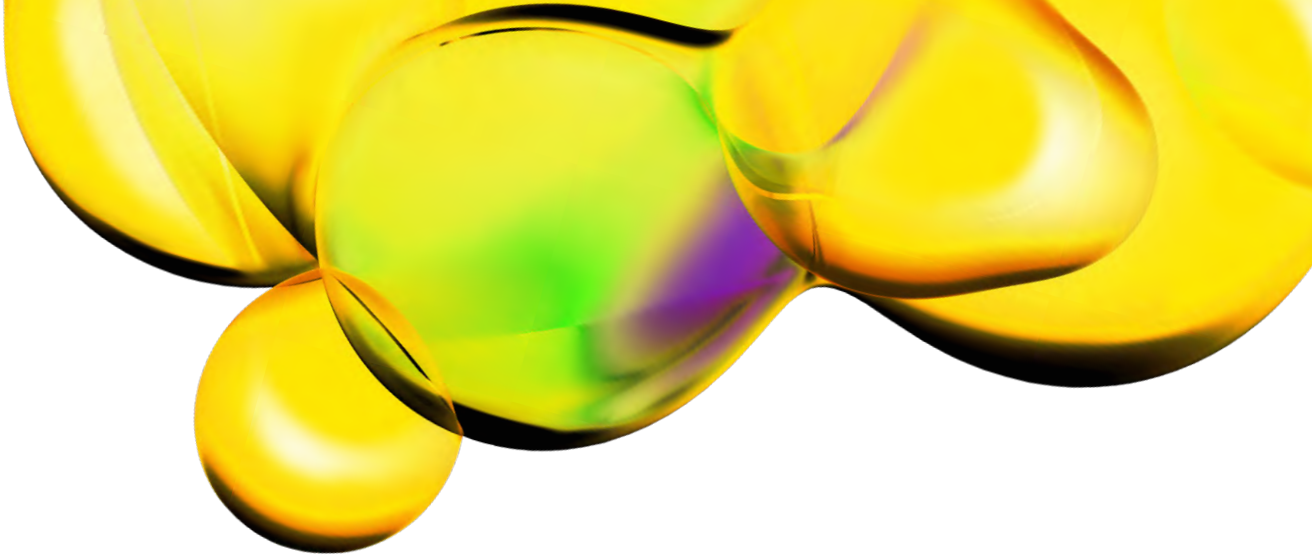
	HTRF Ratio [1]	Delta Ratio [2]	CV [3]
Standard 0 – Negative control	980	0	2.0%
Standard 1 – 11.7 ng/mL	1505	525	1.8%
Standard 2 – 23.4 ng/mL	1994	1014	4.6%
Standard 3 – 46.9 ng/mL	3074	2094	1.8%
Standard 4 – 93.8 ng/mL	4971	3991	1.7%
Standard 5 – 187.5 ng/mL	8257	7277	2.3%
Standard 6 – 375 ng/mL	12854	11874	0.7%
Standard 7 – 750 ng/mL	16227	15247	0.9%



GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS AND LYSATE PREPARATION FREQUENTLY ASKED QUESTIONS / TROUBLESHOOTING PARAMETERS

Using adherent cells, allow time for your cells to recover after plating.	Allow cells to regain full signaling capacity by plating them at least 6 hours before starting the pharmacological treatment.
Depending on the pathway, a serum starving step may be essential to reduce the basal level activity. This step should be optimized case-by-case.	Advice on cell culture conditions prior to using the HTRF kit: - For adherent cells Before treating the cells with compounds, remove culture media from the plate and replace it with serum-free media before incubating from 2 hours up to overnight at 37°C. - For suspension cells The starvation step should be carried out in the flask. Harvest cells by centrifugation and re-suspend cells at a suitable cell density in serum-free media. Incubate from 2 hours up to overnight at 37°C.
Generation of lysates	Ensure that the lysates used for the assay have been generated by using the HTRF® lysis buffer provided in the kit. Lysates generated with HTRF® buffers can be used in other technologies, like Western Blot. The lysis buffer is effective for creating cell extract under non denaturing conditions from both plated cells and cells pelleted from suspension cultures.
Using the two-plate assay manual, a low signal can often be improved by adjusting lysis volumes.	In most cases, a typical adherent cell line grown in 96-well plates is readily detected in a lysis volume of 50 µL. However, the lysis volume can be adjusted from 25 µL to 200 µL.
Using an improper cell density can induce poor sensitivity and low signal.	Check that the cell density is correct. Too high or low cell numbers can affect assay performances.
Parameters such as cell density, stimulation time, and lysis incubation time should be optimized for each cell line used.	The assay can be used for many adherent and non-adherent cell types, including transfected cell lines and primary cells. However, the expression and phosphorylation of the readout of interest can vary from one cell line to another. Depending on the type of treatment, and the temperature, the stimulation time can vary widely. Because of this, we recommend a time course study to determine the optimal compound incubation time. Depending of the nature of your cells, lysis time may vary from 30' to 1h. Because of this, we also recommend determination of the optimal time.
Fluorescence reading	Using an inappropriate setup may seriously impair the results. For information about HTRF® compatible readers and for setup recommendations, please visit our website at: www.revvity.com
Batch production of cell lysates: example of T175 flask	General lab work - prior using HTRF® kit: Day 1: Dispense 3 million cells in T175cm2, add 25 mL of cell culture complete medium and incubate 3 days at 37°C, 5% CO2. Day 4: cell stimulation Remove cell culture medium by aspiration, wash once (do not detach the cells), add 5 mL of compound (1x) diluted in medium and incubate at 37°C, 5% CO2, for the optimized time. Day 4: cell lysis Remove stimulation medium, wash once (do not detach the cells), add 3 ml of 1X HTRF® lysis buffer for 30 min at room temperature under orbital shaking. Transfer the cell lysate to a 15 mL vial, centrifuge the vial, collect the supernatant (soluble fraction), dispense into aliquots and store aliquots at -60°C or below. For long term conservation, lysates should be stored in liquid nitrogen.

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.
This product contains material of biologic origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact.



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