MANUAL

Technology: HTRF™ Epigenetics

HTRF EPIgeneous™ Pan-Acetyl Histone H4 cellular Detection Kit

Part number	62H4PANACPAE	62H4PANACPAD
Test size	500 tests	10,000 tests

Storage: ≤-60°C

Version: 01 **Date:** January 2026

ASSAY PRINCIPLE

This EPIgeneous™ Pan-Acetyl Histone H4 cellular assay is intended for the simple, rapid and direct detection of endogenous levels of Pan-Acetyl Histone H4 mark in cells. The acetylation on Lysines 5, 8, 12 and 16 of on histone H4 is detected in a sandwich assay format using 2 different specific antibodies, one labelled with Eu Cryptate (donor) and the second 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). One reagent binds to Histone H4 and the other binds to acetyl K5-K8-K12-K16 Histone H4, thereby generating FRET. The specific signal modulates positively in proportion to acetylation on Lysines 5, 8, 12 and 16.

The assay can be run under a two-plate assay protocol, where cells are plated, (stimulated) and lysed in the same culture plate and then transferred to the assay plate for the detection of Pan-Acetyl Histone H4 by HTRF™ reagents. This protocol enables the cells' viability and confluence to be monitored. It can also be further streamlined to a one-plate assay protocol. Detection of Pan-Acetyl Histone H4 with HTRF™ reagents is performed in a single plate used for plating, stimulation and detection. No washing steps are required. This protocol, HTS designed, allows miniaturization while maintaining HTRF™ quality.

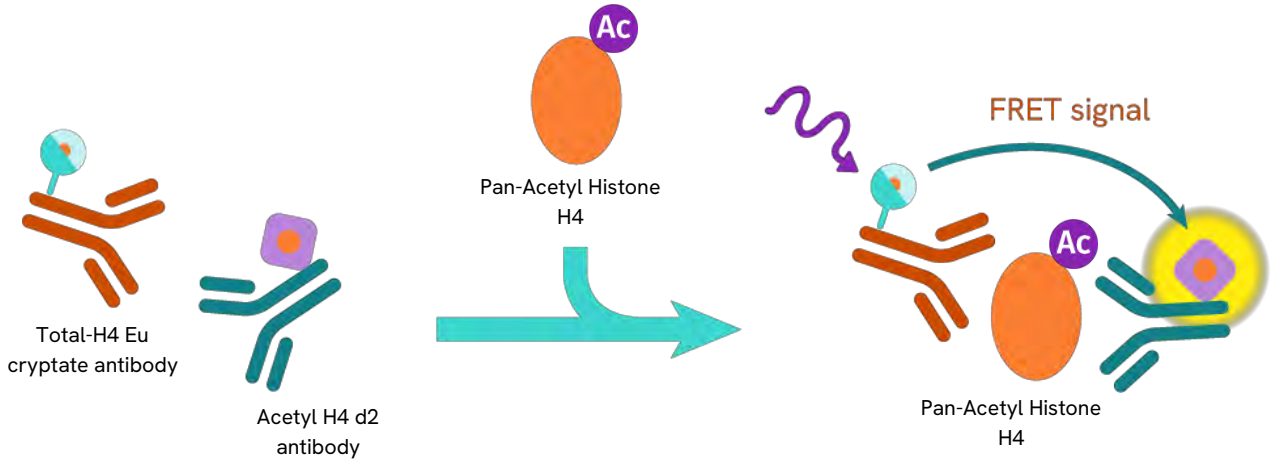
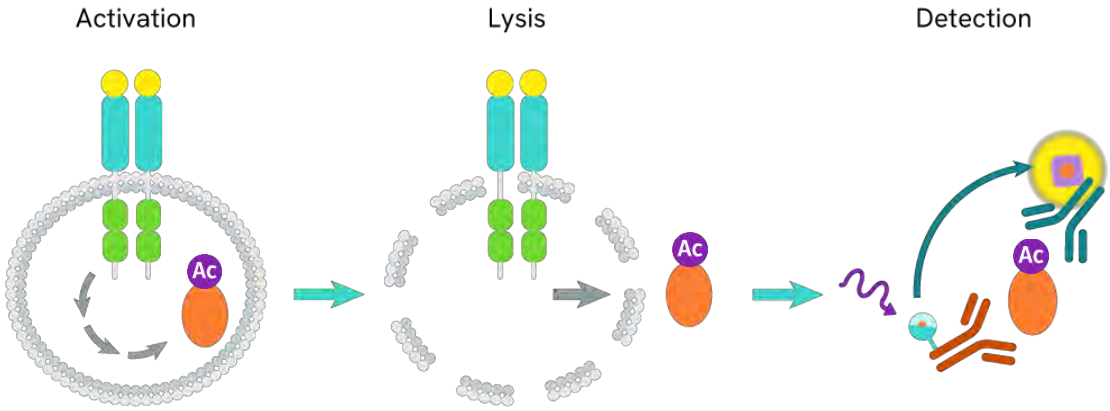


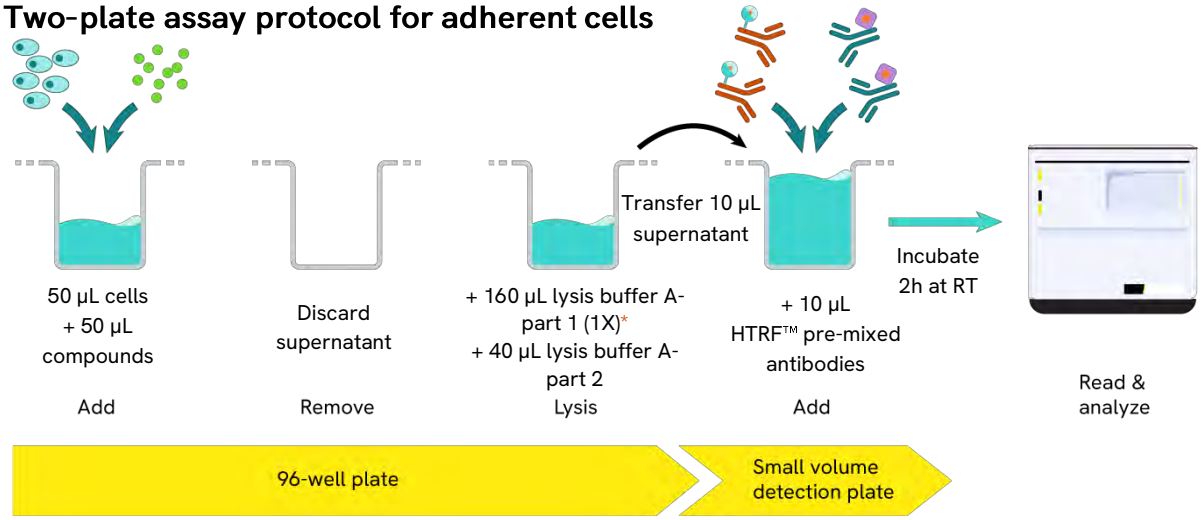
Figure 1: Principle of HTRF sandwich assay.

For tissue derived samples, please refer to the technical note: "Optimize your HTRF™ cell signaling assays on tissues". Technical support team can help you to set-up this protocol or another one. Please contact us.

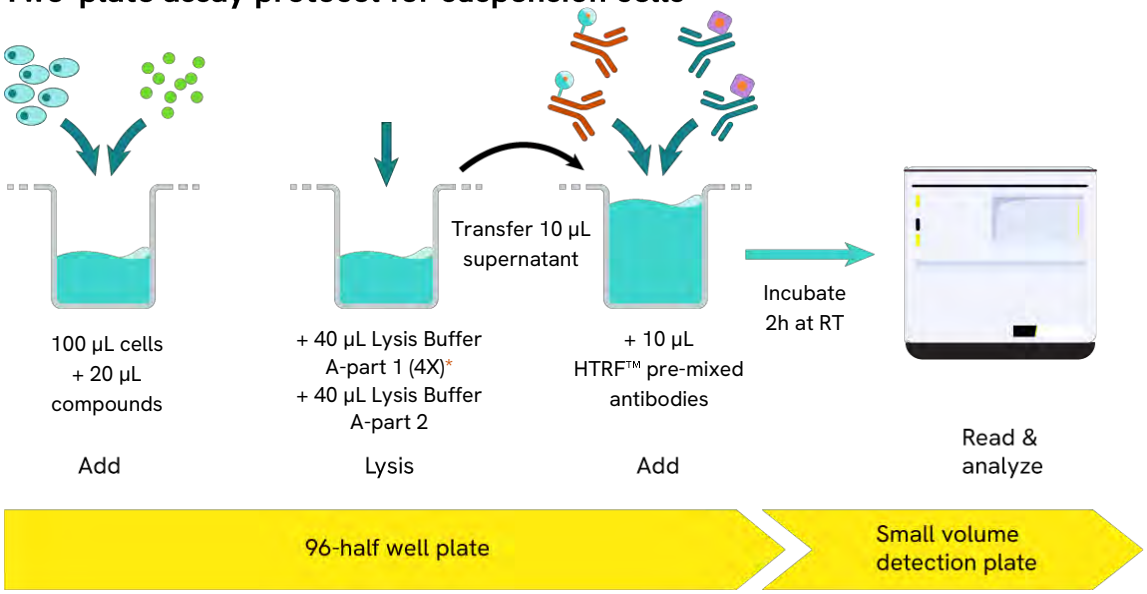
PROTOCOL AT A GLANCE



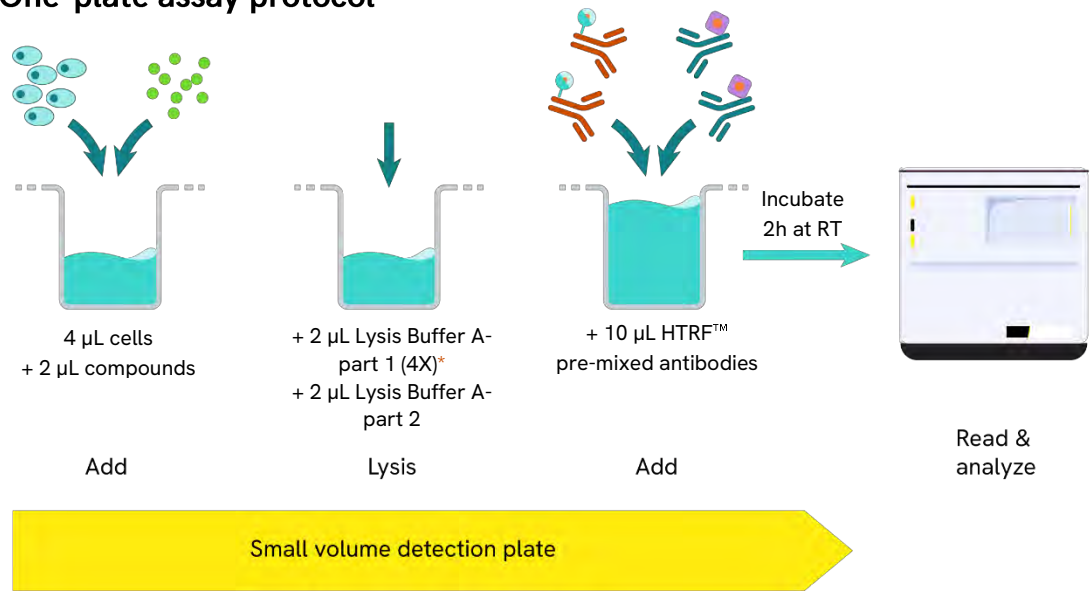
Two-plate assay protocol for adherent cells



Two-plate assay protocol for suspension cells



One-plate assay protocol



* Depending on cell lines used, volume of lysis should be optimized.
Depending on cell lines used, it can be necessary to dilute the cell lysate to ensure samples are within the assay linear range

For HTRF certified reader

For more information about HTRF™ compatible readers and for set-up recommendations, please visit our website.

MATERIAL PROVIDED

KIT COMPONENTS	STORAGE	500 TESTS			10,000 TESTS		
Control lysate (ready-to-use) (Spare part #62H4PANACTDA)	≤-60°C		green cap	1 vial-150 µL		green cap	3 vials-150 µL
H4 Pan-Acetyl Eu Cryptate Antibody (50X)	≤-16°C		red cap	1 vial-50 µL		red cap	1 vial-1 mL
H4 Pan-Acetyl d2 antibody (50X)	≤-16°C		blue cap	1 vial-50 µL		blue cap	1 vial-1 mL
EPIgeneous™ lysis buffer A-part 1 (4X) (Spare part #62EL1FDD/H)	≤-16°C		white cap	1 vial 25 mL		white cap	3 vials 170 mL
EPIgeneous™ lysis buffer A-part 2 (Spare part #62EL1FDD/H)	≤-16°C		red cap	1 vial 25 mL		red cap	3 vials 170 mL
Cellular histone detection buffer	≤-16°C		transparent cap	1 vial 6 mL		white cap	1 vial 120 mL

* Amounts of reagents provided are sufficient for generating 200 µL of cell lysate per well.
** The Detection Buffer is used to prepare working solutions of acceptor and donor reagents.

Purchase separately

96-well or 384-well small volume (SV) detection microplates - For more information about microplate recommendations, please visit our website.

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 protocol 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/RECONSTITUTION
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

*For Antibodies, Protein, Standard & control lysate, 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 10µL. Volume of control lysate aliquots should not be under 20µL.

REAGENT PREPARATION

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.

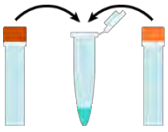
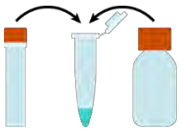
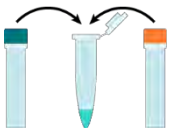
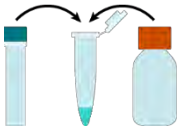
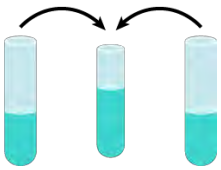
To prepare working control lysate solution

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 and Eu Cryptate-antibodies will impair the assay's quality. Be careful, as working solution preparation for antibodies may differ between the 500 and 10,000 tests data point kit.

Antibody working solutions are stable for 2 days at 2-8°C. Dilute the antibodies with detection buffer. In practice:

500 TESTS KIT	10,000 TESTS KIT
Pan-Acetyl H4 Eu cryptate antibody	
1 volume Eu Cryptate-antibody  49 volumes detection buffer	1 volume Eu Cryptate-antibody  49 volumes detection buffer
Dilute 50-fold the frozen stock solution with detection buffer e.g add 2.45 ml of detection buffer to the 0.05 ml of Eu Cryptate-antibody stock solution.	Dilute 50-fold the frozen stock solution with detection buffer e.g add 49 ml of detection buffer to the 1 ml of Eu Cryptate-antibody stock solution.
Pan-Acetyl H4 d2 antibody	
1 volume d2 antibody  49 volumes detection buffer	1 volume d2 antibody  49 volumes detection buffer
Dilute 50-fold the frozen stock solution with detection buffer e.g add 2.45 ml of detection buffer to the 0.05 ml of d2-antibody stock solution.	Dilute 50-fold the frozen stock solution with detection buffer e.g add 49 mlof detection buffer to the 1 ml of d2-antibody stock solution.
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 ofEu Cryptate-antibody solution.	 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 ofEu Cryptate-antibody solution.

To prepare lysis buffer

Make sure that the lysate has been generated by using the kit reagents.

Be aware that whatever the protocol chosen, two buffers are necessary for the lysis step.

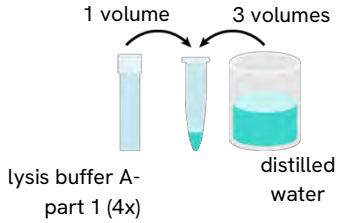
Lysis buffer A-part 1 4X for two-plate assay protocol on suspension cells & one-plate assay protocol

For these assays, the buffer is ready to use.

Lysis buffer A-part 1 1X for two-plate assay protocol on adherent cells

Determine the amount of lysis buffer A-part 1 1X needed for the experiment. Each well requires generally 160 µL of lysis buffer A-part 1 1X. In practice:

TWO-PLATE ASSAY PROTOCOL ON ADHERENT CELLS
500 TESTS KIT & 10,000 TESTS KIT
Preparation of Lysis buffer A-part 1 1X

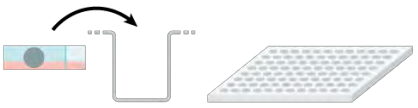
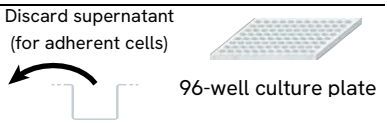
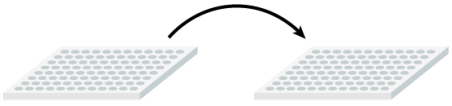
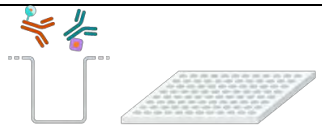


Dilute the "lysis buffer A- part 1 4X" 4-fold with distilled water to prepare lysis buffer 1X. e.g. take 1.25 mL of lysis buffer 4X and add it to 3.75 mL of distilled water. Mix gently.

Lysis buffer A-part 2 for two-plate & one-plate assay protocols on suspension and adherent cells

Lysis buffer A-part 2 is used for all protocols and is ready to use.

TWO-PLATE ASSAY PROTOCOL

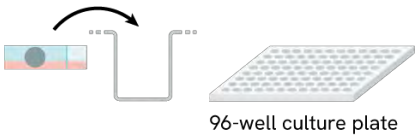
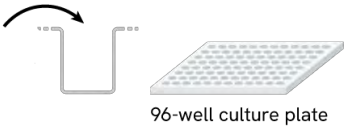
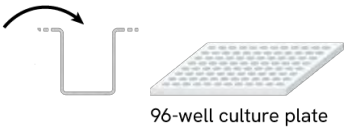
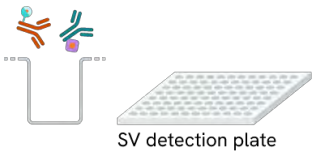
	GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS PREPARATION		
	FOR ADHERENT CELLS	**FOR SUSPENSION CELLS**	
1	Plate 50 µL of cells in 96-well tissue-culture treated plate in appropriate growth medium and incubate overnight, at 37°C in CO2 atmosphere.	Plate 100 µL of cells in 96 half-well plate in yourappropriate medium.	 96-well culture plate
	Cell seeding densities of 6,25-50K cells/well are generally sufficient for most cell lines, but optimization of cell seeding densities is recommended. Depending on receptor a starving step with serum-free medium could be essential.		
2	Dispense 50 µL of compound (2X) diluted in cellculture serum-free medium.	Dispense 20 µL of compound (6X), diluted in yourappropriate medium.	 96-well culture plate
	For most compound, incubation time is between 1 and 20 hours at 37°C. We recommend a time course study to determine the optimal stimulation time.		
3	Remove carefully 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
	Pan-Acetyl H4 DETECTION USING HTRF KIT		
	FOR ADHERENT CELLS	**FOR SUSPENSION CELLS**	
4	Immediately add 160 µL of lysis buffer A-part 1 (1X) and incubate for 45 minutes at room temperature under shaking. After incubation add 40 µL of Lysis Buffer A-part 2.	After desired incubation, add 40 µL of lysis buffer A-part 1 (4X) and incubate for 45 minutes at room temperature under shaking. After incubation add 40 µL of Lysis Buffer A-part 2.	 96-well culture plate
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 theassay 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 2h at room temperature. Set up your reader for Eu^{3+} Cryptate and read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF™ reader.		 SV detection plate

Standard protocol for two-plate assay protocol in 20µL final volume (after lysis step)

		NON TREATED CELL LYSATE	TREATED CELL LYSATE	CONTROL LYSATE	NEGATIVE CONTROL
Step 1		Dispense 10 µL of non treated cell lysate	Dispense 10 µL of treated cell lysate	Dispense 10 µL of control lysate	Dispense 8 µL of Lysis buffer A-part1 1X + 2 µL of Lysis buffer A-part2
Step 2		Add 5 µL of Pan-Acetyl H4 d2 antibody working solution to all wells			
Step 3		Add 5 µL of Pan-Acetyl H4 Eu cryptate antibody working solution to all wells			
Step 4		Cover the plate with a plate sealer. Incubate 2h at room temperature.			
Step 5		Remove the plate sealer and read on an HTRF compatible reader			

The Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

ONE PLATE ASSAY PROTOCOL

	GENERAL LAB WORK PRIOR USING HTRF KIT: CELLS PREPARATION	
1	Plate 4 µL of cells in a small volume (SV) white detection plate in your appropriate medium. Cell seeding densities of 6,25-50K cells/well are generally sufficient for most cell lines, but optimization of cell seeding densities is recommended. Depending on receptor a starving step with serum-free medium can be included.	
2	Dispense 2 µL of compounds (3X) diluted in your appropriate medium. For most compound, incubation time is between 1 and 20 hours at 37°C. We recommend a time course study to determine the optimal stimulation time.	
	Pan-Acetyl H4 DETECTION USING HTRF KIT	
3	Add 2 µL of lysis buffer A-part 1 (4X) and incubate for 45 minutes at room temperature under shaking. After incubation add 2 µL of lysis Buffer A-part 2.	
4	Add 10 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer.Cover the plate with a plate sealer. Incubate 2h at room temperature. Set up your reader for Eu3+ Cryptate and read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF™ reader.	

Standard protocol for one-plate assay protocol in 20 µL final volume

			NON TREATED CELL LYSATE	TREATED CELL LYSATE	NEGATIVE CONTROL	CONTROL LYSATE
General lab work	**Step 1**		Dispense 4 µL of cells		-	
	Step 2		Add 2 µL of your appropriate medium	Add 2 µL of compound (3X)	Add 6 µL of your appropriate medium	Dispense 10 µL of controllysate
Pan-Acetyl H4 Detection Steps	**Step 3**		Add 2 µL of Lysis buffer A-part1 1X/45 min at TA under shaking + 2 µL of Lysis buffer A-part2			-
	Step 4		Add 5 µL of Pan-Acetyl H4 d2 antibody solution to all wells			
	Step 5		Add 5 µL of Pan-Acetyl H4 Eu cryptate antibody solution to all wells			
	Step 6		Cover the plate with a plate sealer. Incubate 2h at room temperature.			
	Step 7		Remove the plate sealer and read on an HTRF compatible reader			

Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

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

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

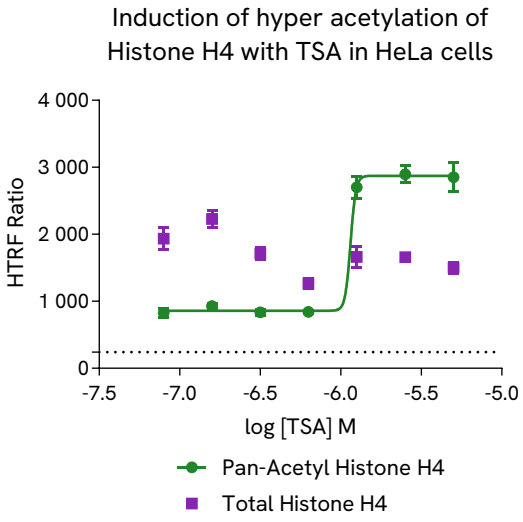
For more information about data reduction, please visit our website.

RESULTS

These data should be considered only as an example. Results may vary from one HTRF™ compatible reader to another. The curves are drawn up by plotting HTRF™ Ratio versus the log [compound] concentrations.

The curves are drawn up by plotting HTRF™ Ratio versus the log [compound] concentrations.
Results on HeLa cells plated at 12,500 cells per well, using the two-plate assay protocol for adherent cells. Cells were treated with increasing concentrations of Trichostatin A (TSA) for 18h. Cells were then lysed with 160 µL of EPIgeneous Lysis Buffer A-part 1 and incubate for 45 minutes at room temperature under shaking. After incubation 40 µL of EPIgeneous Lysis Buffer A-part 2 were added.
10 µL of lysates (1/4 dilution) were transferred in a first plate to detect Pan-Acetyl Histone H4, and 10 µL of lysates (1/4 dilution) were transferred in a second plate to detect Total Histone H4 using the HTRF Total-Histone H4 assay - Cat #62H4TPAE, #62H4TPAD. The HTRF signal was recorded with an Envision Nexus reader after a 2h-incubation.

		Pan-Acetyl H4		Total H4	
[TSA] (µM)	Log([TSA]) (M)	Mean HTRF Ratio	CV	Mean HTRF Ratio	CV
5	-5.3	2850	7.8%	1500	5.6%
2.5	-5.6	2895	4.4%	1658	3.6%
1.3	-5.9	2703	6.2%	1662	9.2%
0.6	-6.2	843	2.3%	1265	6.3%
0.3	-6.5	835	4.6%	1713	5.3%
0.2	-6.8	928	4.4%	2227	5.9%
0.1	-7.1	829	7.6%	1934	8.7%
Negative		242	0.4%	226	1.2%



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 beforestarting the pharmacological treatment.
Depending on the pathway, a serum starving step could be essential toreduce the basal level activity. This step should be optimized case-by- case.	Advice on cell culture conditions prior using 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 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 hoursup to overnight at 37°C.
Using the two-plate assay protocol, a low signal can often be improvedby adjusting lysis volumes.	In most cases, a typical adherent cell line grown in 96-well plates is readily detected ina lysis volume of 200µL. However, the lysis volume can be adjusted from 25 µL to 200µL.
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 assayperformances.
Parameters such as cell density, stimulation time and lysis incubationtime 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 phosphorylationof the readout of interscan vary from one cell line to another. Depending on the type of treatment, and the temperature, the stimulation time can vary widely. Becauseof 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 ofthis, we also recommend determination of the optimal time.
Fluorescence reading	Using an inappropriate set-up may seriously impair the results. For information about HTRF™ compatible readers and for set-up recommendations, please visit our website
Assaying for multiple targets from a single lysate.	The two-plate assay protocol indicates the use of 10 µL of lysate per well, whereas the 96-well cell culture microplate would generate 200µL (or more) of lysate. Therefore, a typical cell lysate can be assayed for many targets, given that temporal and expression level constraints can vary from one target to another.
Batch production of cell lysates example of T175 flask	General lab work - prior using Pan-Acetyl H4 HTRF™ kit: Day1: Dispense 8 million cells in T175cm2, add 25 mL of cell culture complete medium and incubate 2 days at 37°C, 5% CO2. Day3: cell stimulation Remove cell culture medium by aspiration, wash once (do not detach the cells), add 5 mL of agonist (1x) diluted in FCS free medium and incubate at 37 ° C, 5% CO_2, for the optimized time. Day3: cell lysis Remove stimulation medium, wash once (do not detach the cells), add 2.6 ml of lysis buffer A-part 1 (1X) and incubate for 45 minutes at room temperature under shaking. After incubation, add 40 µL of lysis Buffer A-part 2. Transfer the cell lysate to a 15 mL vial, centrifuge 10 min, 2400 rcf at RT, recover cell lysate supernatant and store aliquots at -60°C or below. For long term conservation, aliquots should be stored in liquid nitrogen.

REACH European regulations and compliance
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. 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.

Manufactured by Cisbio Bioassays
172 rue de la Cèze, Parc Marcel Boiteux
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