TOTAL H3 CELLULAR ASSAY

10,000 TESTS

PROTOCOL

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

Storage temperature: ≤-60°C

Packaging details :

	384-well low volume plate (20 µL)
62NH3PAD	10,000 tests

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Product information:

Document reference : 62NH3PAD rev04 (Sep. 2020)

1. Assay description

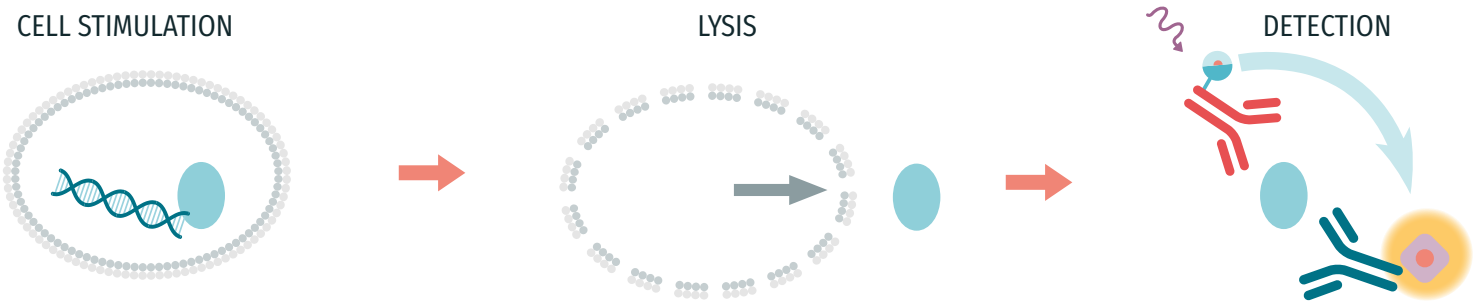
This EPIgeneous™ Total H3 cellular assay is intended for the simple, rapid and direct detection of endogenous levels of Histone H3 Total in cells. Histone H3 is detected in a sandwich assay format using 2 different specific antibodies, one labelled with Eu Cryptate (donor) and the second with d2 (acceptor).

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). The specific signal modulates positively in proportion to H3 Total presence.

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 H3 Total by HTRF® reagents. This protocol allows the cells viability and confluence to be monitored. Moreover, H3 Total kit has been designed to be run in parallel of EPIgeneous™ specific methyl mark cellular assay. The same stimulated lysate can be split and assessed for both the histone methylation level at a specific position and the total expression level, as stimulating compounds can impact both.

It can also be further streamlined to a one-plate assay protocol. Detection of Total H3 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.

2. Assay Principle



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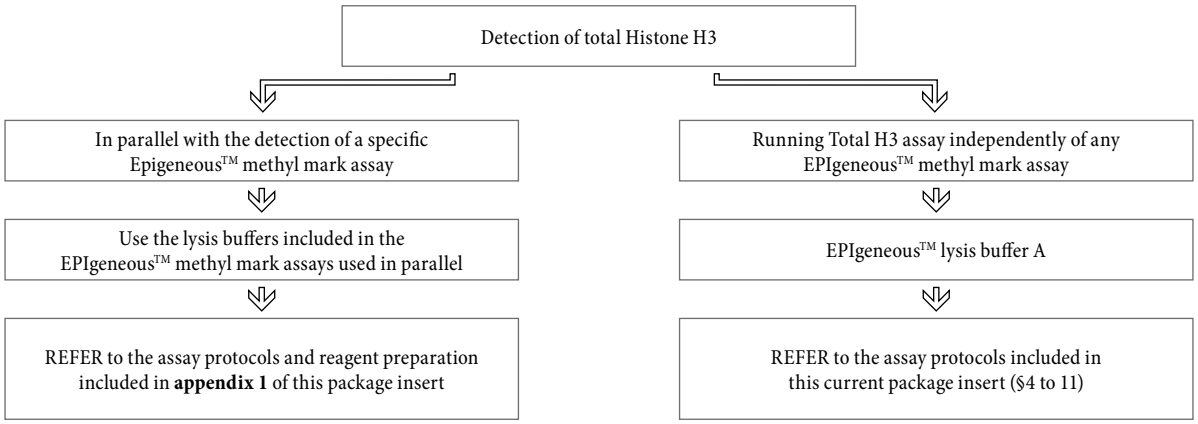
3. Intended use and buffer conditions

As shown below, the Total H3 Cellular assay is designed to be run either as a stand alone quantification or in parallel with another EPIgeneous™ methyl mark specific assay.
Depending on the use of the kit, different lysis buffer conditions will apply - see below and in §4, and in appendix 1 of this document.



4. HTRF® reagents

		Nb of vials	Volume per vial	Storage
Anti-H3-Eu Cryptate Antibody	red cap	1	1 mL	≤-16°C
H3 d2 Antibody (Total H3 kit)	blue cap	1	1 mL	≤-16°C
Total H3 Cellular Control Lysate (Ready to use)	green cap	3	150 µL	≤-60°C
Cellular Histone Detection Buffer (ready-to-use)	Transparent cap	1	120 mL	≤-16°C

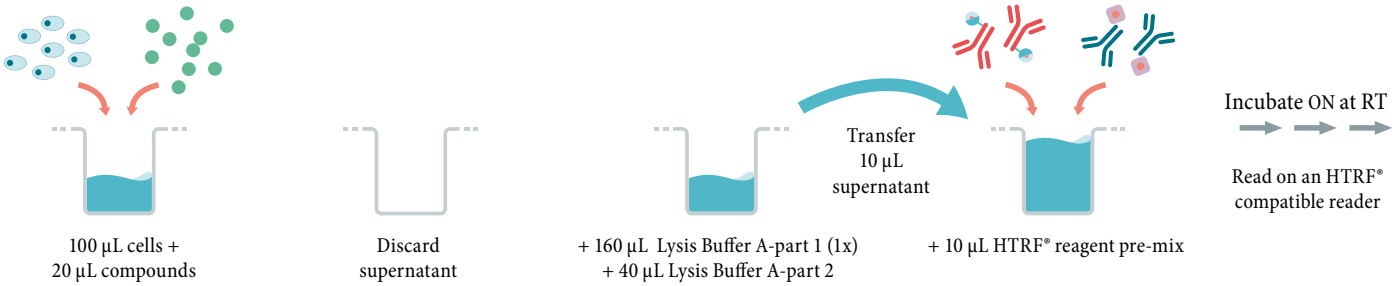
When running Total H3 assay independently of any EPIgeneous™ methyl mark assay, lysis buffer A has to be ordered separately as an additional reagent.

If using this Total Histone H3 assay in parallel with the detection of a specific EPIgeneous™ methyl mark assay, please refer to appendix 1 of this document.

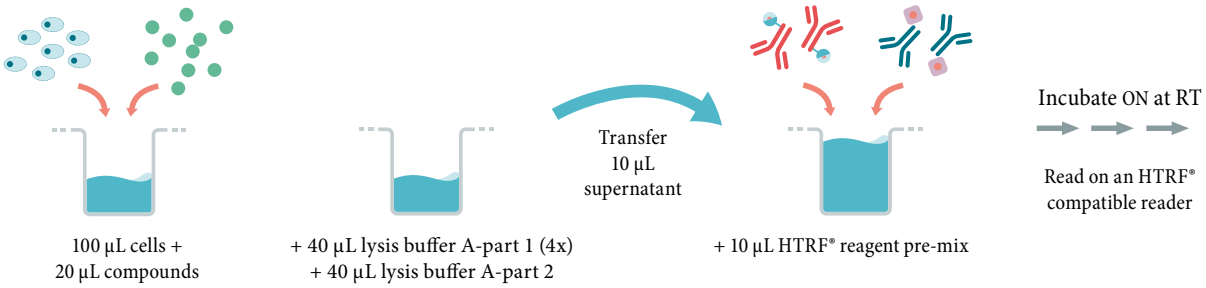
	EPIgeneous™ Lysis buffer A – additional reagent, not provided		
		Nb of vials	Volume per vial
EPIgeneous™ Lysis buffer A-part 1 (stock solution (4x))	White cap	3	170 mL
EPIgeneous™ Lysis buffer A-part 2 (ready to use)	Red cap	3	170 mL

5. Assay protocols using EPIgeneous™ lysis buffer A

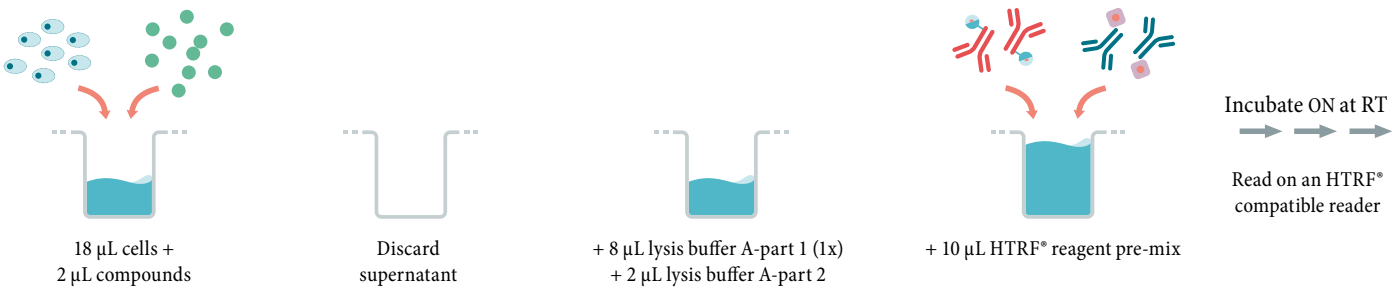
➥ *Two-plate assay protocol for adherent cells*



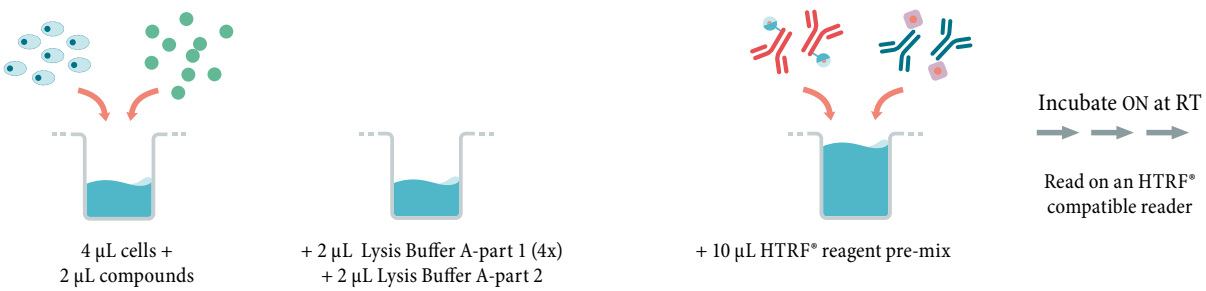
➥ *Two-plate assay protocol for suspension cells or adherent cells kept in medium for lysis*



➥ *One-plate assay protocol for adherent cells*



➥ *One-plate assay protocol for suspension cells and adherent cells kept in medium for lysis*



6. Storage stability

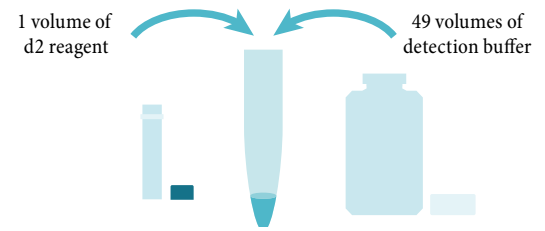
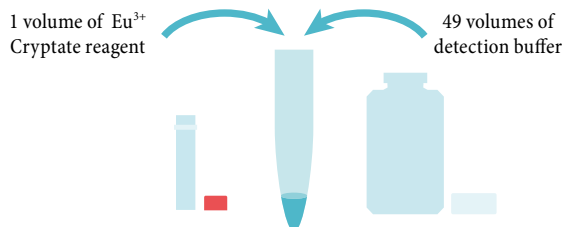
All reagents should be stored frozen until needed.
To avoid freeze/thaw cycles, aliquot stock solutions into disposable plastic vials for storage at -20°C or below.
Thawed lysis and detection buffers can be stored at 2-8°C.

7. Reagent preparation

Allow all reagents to thaw before use.
We recommend centrifuging the vials gently after thawing, before pipeting the stock solutions.
Prepare the working solutions from stock solutions by following the instructions below.
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.

7.1. Preparation of reagent working solutions

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Eu Cryptate-reagents will impair the assay's quality. Dilute the reagents with detection buffer. In practice:

H3 d2 Antibody	H3 Eu Cryptate Antibody
	
Dilute the frozen stock solution 50-fold with detection buffer: e.g. add 2,450 µL of detection buffer to the 50 µL of reagent stock solution (in a different vial).	Dilute the frozen stock solution 50-fold with detection buffer: e.g. add 2,450 µL of detection buffer to the 50 µL of reagent stock solution (in a different vial).

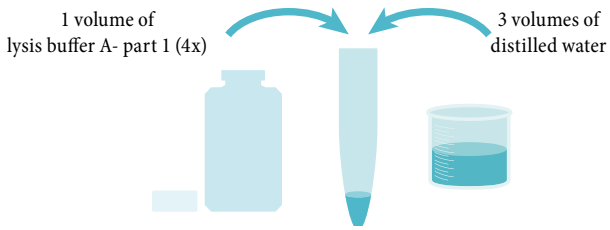
7.2. Preparation of lysis buffer

Make sure that lysate has been generated by using the EPIgeneous™ lysis buffer.
Be aware that whatever the protocol chosen, two buffer components are necessary for the lysis step.

➥ *Lysis buffer A - part 1 (1x) for two-plate and one-plate assay protocols on adherent cells*

Prepare the required amount of lysis buffer A-part 1 (1x) before running the assay.
Determine the amount of lysis buffer A-part 1 needed for the experiment:
- For the two-plate assay protocol, each well requires 160 µL of lysis buffer A-part 1 (1x).
- For the one-plate assay protocol, each well requires 8 µL of Lysis buffer A-part 1 (1x).

In practice:

Preparation of Lysis buffer A-part 1 (1x)

Dilute the lysis buffer A-part 1 (4x) 4-fold with distilled water 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 1 (4x) for two-plate & one-plate assay protocols for suspension cells and adherent cells kept in medium for lysis*

For these assays, the buffer is ready to use.
Determine the amount of lysis buffer A-part 1 (4x) needed for the experiment:
- For the two-plate assay protocol on suspension cells or homogeneous assay with adherent cells, each well requires 40 µL of lysis buffer A-part 1 (4x).
- For the one-plate assay protocol, each well requires 2 µL of lysis buffer A-part 1 (4x).

➥ *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.

8. Two-plate assay protocol step by step

	For adherent cells	For suspension cells and adherent cells kept in medium for lysis	
1	Plate 100 µL of cells in a 96-well tissue-culture treated plate in appropriate growth medium. Incubate 3 to 4h at 37°C in CO_2 atmosphere in order to allow cell adhesion. Optimization of cell seeding densities is required.	Plate 100 µL of cells in a 96-well tissue-culture treated plate, in appropriate growth medium. Optimization of cell seeding densities is required.	 96-well culture plate
2	Dispense 20 µL of compound (6X), diluted in cell culture medium. Incubate the cells with the compound for the required time. We recommend a time course study to determine the optimal incubation time.		 96-well culture plate
3	***Carefully remove supernatant using aspiration.*** Be careful not to touch the cell layer. 	***Do not remove supernatant*** 	
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 pipetting up and down, transfer 10 µL of cell lysate from the 96-well cell-culture plate to a 384-well small volume white plate.		 96-well culture plate 384-well SV plate
6	Add 5 µL of each HTRF® reagent, prepared in the detection buffer. The 2 reagents can also be pre-mixed JUST PRIOR to dispensing, and 10 µL of this pre-mix is added. Cover the plate with a plate sealer. Incubate ON at room temperature. Read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on an HTRF®-compatible reader.		 384-well SV plate
For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: www.cisbio.com/content/htrf-microplate-readers			

➥ *Two-plate assay protocol in 20 µL final volume after lysis step: standard protocol*

	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	10 µL	10 µL	-	10 µL	-
Control lysate	-	-	10 µL		
Lysis buffer A-part 1 (1x)	-	-	-	-	8 µL
Lysis buffer A-part 2	-	-	-	-	2 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-
H3 d2 Antibody	5 µL	5 µL	5 µL	-	5 µL
H3 Eu Cryptate Antibody	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL

* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

9. One-plate assay protocol step by step

	For adherent cells	For suspension cells and adherent cells kept in medium for lysis	
1	Plate 18 µL of cells in a 384-well small volume tissue-culture treated white plate in appropriate growth medium. Optimization of cell seeding densities is required. The use of tissue culture treated plate is mandatory.	Plate 4 µL of cells in a 384-well small volume tissue culture treated white plate in appropriate growth medium. Optimization of cell seeding densities is required.	 96-well culture plate
2	Dispense 2 µL of compound (10X) diluted in appropriate growth medium.	Dispense 2 µL of compound (3X) diluted in appropriate growth medium.	 96-well culture plate
	Incubate the cells with compound for required time. Evaporation can be problematic with cells cultured in microtiter plates for a long incubation time (overnight and more). This evaporation issue must be managed carefully. We recommend a time course study to determine the optimal incubation time. We recommend checking the evaporation issue during incubation.		
3	*Carefully remove supernatant using aspiration.* Be careful not to touch the cell layer. 	*Do not remove supernatant.* 	
4	Add 8µL of lysis buffer A-part 1 (1x) and incubate for 45 minutes at room temperature. After incubation, add 2 µL of lysis Buffer A-part 2 Use the appropriate lysis buffer.	Add 2 µL of lysis buffer A-part 1 (4x) and incubate for 45 minutes at room temperature. After incubation add 2 µL of lysis Buffer A-part 2 Use the appropriate lysis buffer.	 384-well SV plate
5	Add 5 µL of each HTRF® reagent, prepared in the detection buffer. The 2 reagents can also be pre-mixed JUST PRIOR to dispensing and 10 µL of this pre-mix is added. Cover the plate with a plate sealer. Incubate ON at room temperature. Read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on an HTRF®-compatible reader.		 384-well SV plate

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at:
www.cisbio.com/content/htrf-microplate-readers

➥ *One-plate assay protocol in 20 µL final volume: standard protocol for adherent cells*

	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	18 µL	18 µL	-	18 µL	-
Cell culture Medium	2 µL	-	-	2 µL	-
Compound(s)	-	2 µL	-	-	-
	Discarded volume				
Control lysate	-	-	10 µL	-	-
Lysis buffer A-part 1 (1x)	8 µL	8 µL	-	8 µL	8 µL
Lysis buffer A-part 2	2 µL	2 µL	-	2 µL	2 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-
H3 d2 Antibody	5 µL	5 µL	5 µL	-	5 µL
H3 Eu Cryptate Antibody	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL

➥ *One-plate assay protocol in 20 µL final volume: standard protocol for suspension cells and adherent cells kept in medium for lysis*

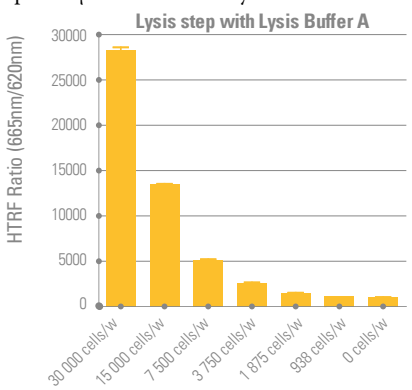
	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	4 µL	4 µL	-	4 µL	-
Control lysate	-	-	10 µL	-	-
Cell culture Medium	2 µL	-	-	2 µL	6 µL
Compound(s)	-	2 µL	-	-	-
Lysis buffer A-part 1 (4x)	2 µL	2 µL	-	2 µL	2 µL
Lysis buffer A-part 2	2 µL	2 µL	-	2 µL	2 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-
H3 d2 Antibody	5 µL	5 µL	5 µL	-	5 µL
H3 Eu Cryptate Antibody	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL

* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

10. Data reduction

This data should be considered as an example only. Results may vary from one HTRF compatible reader to another. The histogram is drawn up by plotting HTRF Ratio versus number of cells seeded in 96-well plate.
Results on SU-DHL-6 cells (various numbers of cells per well), using the two-plate assay protocol for suspension cells. Cells were incubated for 24h before lysis step following the previously described protocol with lysis Buffer A. Transfer of 5 µl cell lysate, back field up to 10 µL with the mix Lysis Buffer mix.

	Lysis Buffer A	
	HTRF Ratio (1)	CV% (2)
30,000 cells/w	28326	0.9
15,000 cells/w	13495	0.4
7,500 cells/w	5124	1.9
3,750 cells/w	2606	2.9
1,875 cells/w	1517	2.1
937 cells/w	1212	0.3
0 cells/w	1019	2.3



HTRF Ratio (1)	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	HTRF Ratio must be calculated for each individual well.
CV% (2)	$\frac{\text{Standard deviation}}{\text{Mean ratio}} \times 100$	The mean and standard deviation can then be worked out from ratio replicates.

For more information about data reduction, please visit our website at: www.cisbio.com/content/signal-treatment-and-analysis

11. How to improve your assay performance

Depending on compound incubation time, medium evaporation can occur in microtiter plate (sv-384-plate)	A sterile breathable sealing membrane plus special lid can be placed on the plate. Sterile warm PBS can be added to all outer and unused wells. (Low base sv-384-plate Greiner # 788073, lid Greiner # 691 161). Check the evaporation issue during incubation.
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, specific lysine mark methylation can vary from one cell line to another. Be sure to first determine the best cell concentration that is suited to the required stimulation time. Too high or low cell numbers can affect detection of methyl mark. Be carefull of hook effect. Some compounds are described as active after long incubation time. Stimulation time needs to be optimized.
EPIgeneous™ Total H3 normalization assay	The two-plate assay protocol recommends the use of 10 µL of lysate per well, whereas the 96-well cell culture microplate would use 160 µL or 200 µL of lysis buffer per well (depeding on lysis buffer used). Therefore, a typical cell lysate can also be assayed for total H3 level (using EPIgeneous™ Total H3 cellular assay) in order to measure the compound effect on Total Histone H3 level. Depending on the cell line used for the assay, Total H3 kit might be more or less sensitive compared to specific methyl mark kit. Please adapt in consequence the volume of cell lysate transferred in consequence (adjusted with Mix lysis Buffer A, see protocol), or adapt the number of cells seeded.
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 at: *www.cisbio.com/content/htrf-microplate-readers*
Using adherent cells, allow time for your cells to recover after plating	Allow cells to recover by plating them at least 3-4 hours before starting the pharmacological treatment.
Limit the DMSO percentage given with compounds	Please, note that concentration above 0.5% DMSO (during compound treatment) will impair assay performances.
Generation of lysates	Ensure that the lysates used for the assay have been generated by using the HTRF lysis buffer provided in the kit.

To obtain additional information or support, please contact your technical support team (www.cisbio.com/contact-us).

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This product contains material of biological 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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APPENDIX - USING H3 CELLULAR ASSAY (#62NH3PAE) IN COMBINATION WITH METHYL MARK-SPECIFIC CELLULAR ASSAY

APPENDIX

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

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Product information:

1. Introduction

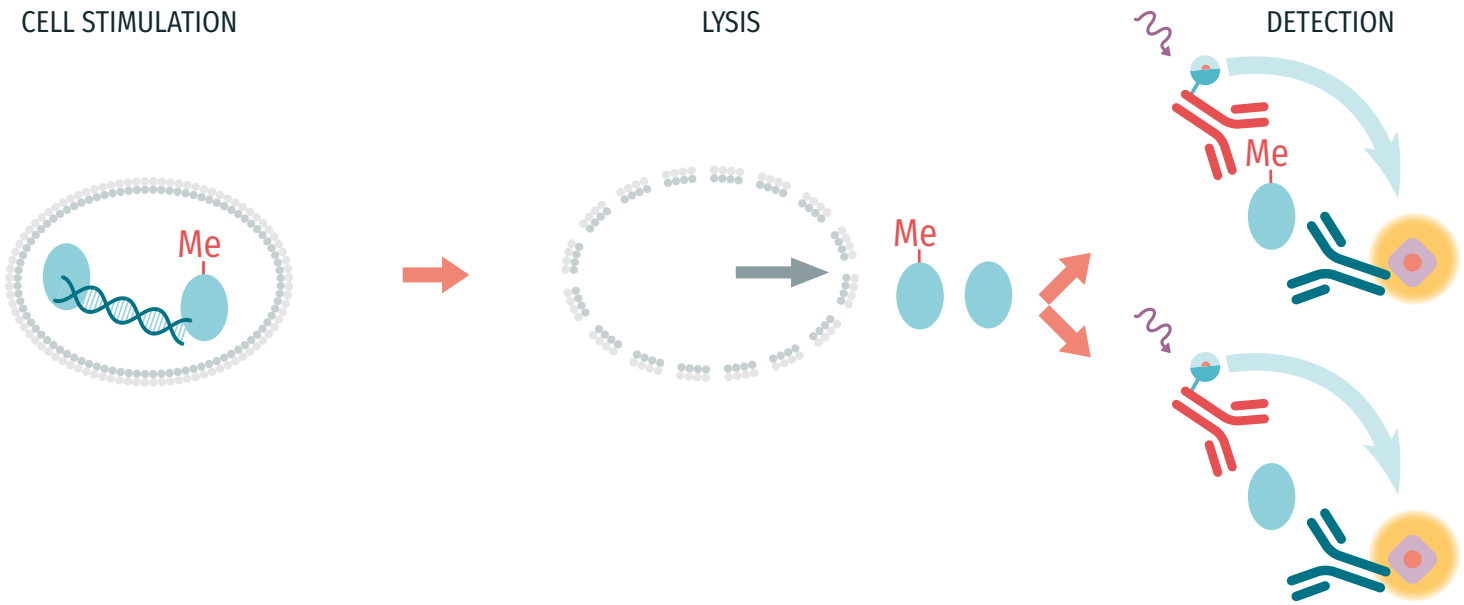
The study of histone methylation under a cell-based format implies the stimulation of specific cells. Several hours or even days may be necessary to properly stimulate cells with epigenetic enzyme activating compounds. Such a lengthy stimulation may affect histone expression itself, hence the need for normalizing the methylation detection vs the histone expression level, i.e. methyl mark assessment / total histone assessment.

This appendix describes how to combine the use of total H3 cellular assay (#62NH3PAE / (#62NH3PAD) and the following EPIgeneous™ cellular assays for the detection of specific methyl marks:

- H3K4Me2 cellular assay (#62KA2PAE / 62KA2PAD) for dimethylation of Lysine 4 on Histone H3
- H3K27Me3 cellular assay (# 62KC3PAE / 62KC3PAD) for trimethylation of Lysine 27 on Histone H3
- H3K36Me2 cellular assay (# 62KD2PAE / 62KD2PAD) for dimethylation of Lysine 36 on Histone H3

As per the graphics below, the experimental procedure includes 3 main steps:

- Cell stimulation which triggers methylation enzymes
- Cell lysis with a buffer specific to the methyl mark investigated
- Using the same cell lysate, measurement of i) histone methylation level and ii) total histone level



2. Using the right lysis buffer

When the total H3 assay is used on its own, the lysis buffer A provided with the kit must be used (see section 3 & 4 of the kit documentation). Buffer A must be ordered separately as an additional reagent.

Caution! When the total H3 assay is to be combined with an EPIgeneous™ methyl mark specific cellular assay for normalization purposes, the lysis buffer used must be that provided in the latter. Each buffer composition differs from one assay to another as shown in the table below:

Cellular Assay	Lysis buffer combination
H3K4Me2 (#62KA2PAE / 62KA2PAD)	Buffer C part-1&2
H3K27Me3 (# 62KC3PAE / 62KC3PAD)	Buffer A part-1&2
H3K36Me2 (# 62KD2PAE / 62KD2PAD)	Buffer B + buffer C part-1&2

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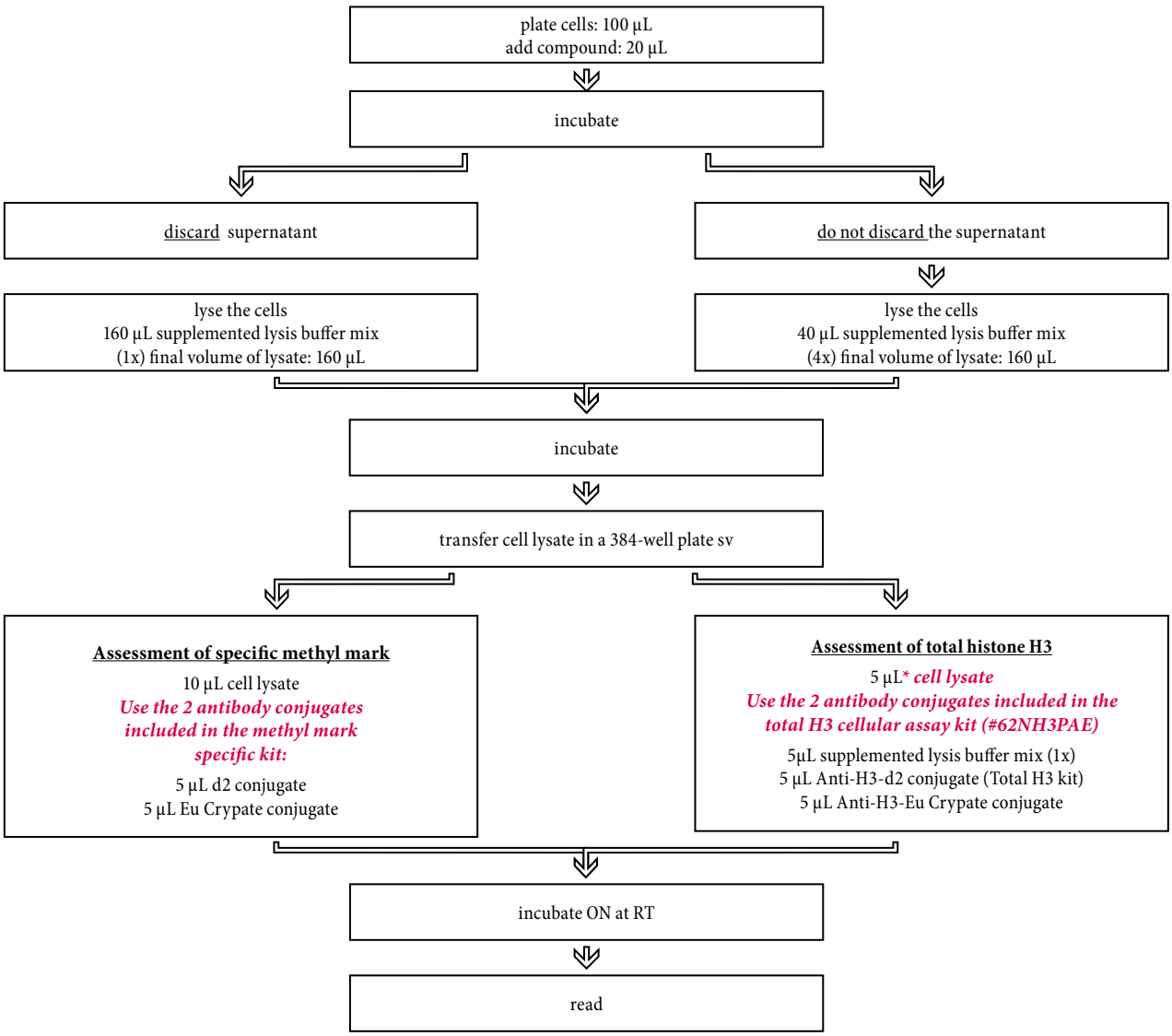
3. Two-plate assay protocols

Cell seeding, cell stimulation, cell lysis are performed in a single well

The volume of lysate generated in the two-plate assay protocols is sufficient for the detection of both the methyl mark and the total Histone H3.

Use the lysis buffer included in the methyl mark specific assay to be normalized.

Use the specific pairs of antibodies (included in each kit) for the detection.



* Due to the high sensitivity of total Histone H3 assay, we recommend transfering 5 µL of lysate backfilled up to 10 µL with supplemented Lysis buffer mix (1x). Nevertheless, if a higher signal is required, 10 µL of lysate can be used and in this case do not add supplemented lysis buffer mix (1x).

➥ *Two-plate assay protocols step by step*

	For adherent cells	For suspension cells and adherent cells kept in medium for lysis	
1	Plate 100 µL of cells in a 96-well tissue-culture treated plate in appropriate growth medium. Incubate 3 to 4h at 37°C in CO2 atmosphere in order to allow cell adhesion. Optimization of cell seeding densities is required.	Plate 100 µL of cells in a 96-well tissue-culture treated plate, in appropriate growth medium. Optimization of cell seeding densities is required.	
2	Dispense 20 µL of compound (6x), diluted in cell culture medium. Incubate the cells with the compound for the required time We recommend a time course study to determine the optimal incubation time.		
3	***Carefully remove supernatant using aspiration.*** Be careful not to touch the cell layer. 	***Do not remove supernatant*** 	
4	Immediately add 160 µL of supplemented lysis buffer mix (1x) and incubate for 45 minutes at room temperature under shaking.	After desired incubation, add 40 µL of supplemented lysis buffer mix (4x) and incubate for 45 minutes at room temperature under shaking.	
5	After homogenization by pipetting up and down, transfer 10 µL of cell lysate from the 96-well cell-culture plate to a 384-well small volume white plate.		
6	Add 5 µL of each HTRF® conjugate, prepared in the detection buffer. The 2 conjugates can also be pre-mixed JUST PRIOR to dispensing, and 10 µL of this pre-mix is added. *use the conjugates included in each kit:* - for the methyl mark specific assay - and for Total Histone H3 detection (kit#62NH3PAE) Cover the plate with a plate sealer. Incubate ON at room temperature. Read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on an HTRF®-compatible reader.		

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: www.cisbio.com/content/htrf-microplate-readers

➥ *Two-plate assay protocols in 20 µL final volume after lysis step: standard protocol*

	Specific methyl mark detection						Total Histone H3 detection					
	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**		Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**	
Cells	10 µL	10 µL	-	10 µL	-	-	5 µL	5 µL	-	10 µL	-	-
Control lysate	-	-	10 µL	-	-	-	-	-	10 µL	-	-	-
Supplemented lysis mix (4x) (depending on protocol used)	-	-	-	-	4 µL	-	-	-	-	-	4 µL	-
Supplemented lysis mix (1x) (depending on protocol used)	-	-	-	-	-	10 µL	5 µL	5 µL	-	-	-	10 µL
Growth medium	-	-	-	-	6 µL	-	-	-	-	-	6 µL	-
Cellular Histone Detection Buffer	-	-	-	5 µL	-	-	-	-	-	5 µL	-	-
Anti-H3-d2 Conjugate (methyl mark kit)	5 µL	5 µL	5 µL	-	5 µL	5 µL	-	-	-	-	-	-
Anti-Eu Cryptate Conjugate (methyl mark kit)	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	-	-	-	-	-	-
Anti-H3-d2 Conjugate (total H3 kit)	-	-	-	-	-	-	5 µL	5 µL	5 µL	-	5 µL	5 µL
Anti-H3-Eu3 Cryptate Conjugate (total H3 kit)	-	-	-	-	-	-	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL

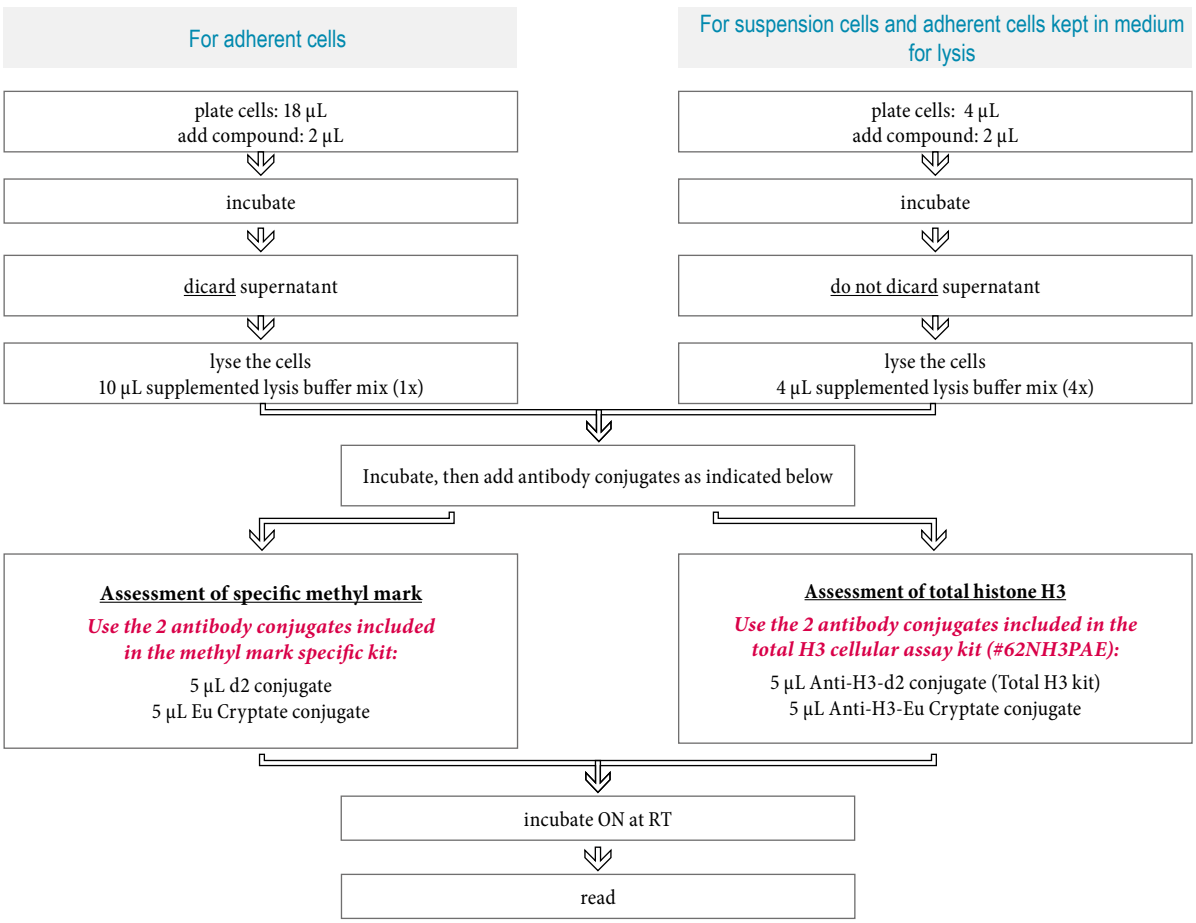
* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

4. One-plate assay protocols

As cell seeding, cell stimulation, cell lysis and cell detection are performed in a single well, the 2 assays (methyl mark and total Histone H3) have to be performed in parallel.

Use the lysis buffer included in the methyl mark specific assay to be normalized.

Use the specific pairs of antibodies (included in each kit) for the detection.



➥ *One-plate assay protocols step by step*

	For adherent cells	For suspension cells and adherent cells kept in medium for lysis	
1	Plate 18 µL of cells in a 384-well small volume tissue-culture treated white plate in appropriate growth medium. Optimization of cell seeding densities is required. The use of tissue culture treated plate is mandatory.	Plate 4 µL of cells in a 384-well small volume tissue culture treated white plate in appropriate growth medium. Optimization of cell seeding densities is required.	
2	Dispense 2 µL of compound (10x) diluted in appropriate growth medium.	Dispense 2 µL of compound (3x) diluted in appropriate growth medium.	
	Incubate the cells with compound for required time. Evaporation can be problematic with cells cultured in microtiter plates for a long incubation (overnight and more). We recommend a time course study to determine the optimal incubation time. We recommend checking the evaporation issue during incubation.		
3	*Carefully remove supernatant using aspiration.* Be careful not to touch the cell layer. 	*Do not remove supernatant* 	
4	Add 10 µL of supplemented lysis buffer mix (1x) and incubate for 45 minutes at room temperature. Use the appropriate lysis buffer. We recommend a time course study to determine the optimal lysis incubation time.	Add 4 µL of supplemented lysis buffer mix (4x) and incubate for 45 minutes at room temperature. Use the appropriate lysis buffer. We recommend a time course study to determine the optimal lysis incubation time.	
5	Add 5 µL of each HTRF® reagent, prepared in the detection buffer. The 2 reagents can also be pre-mixed JUST PRIOR to dispensing, and 10 µL of this pre-mix is added. Use the reagents included in each kit: - for the methyl mark specific assay - and for Total Histone H3 detection (kit#62NH3PAE) Cover the plate with a plate sealer. Incubate ON at room temperature. Read the fluorescence emission at two different wavelengths (665 nm and 620 nm) on an HTRF®-compatible reader.		

For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: ***www.cisbio.com/content/htrf-microplate-readers***

➥ *One-plate assay protocol in 20 µL final volume: standard protocol for adherent cells*

	Specific methyl mark detection					Total Histone H3 detection				
	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	18 µL	18 µL	-	18 µL	-	18 µL	18 µL	-	18 µL	-
Cell culture Medium	2 µL	-	-	2 µL	-	2 µL	-	-	2 µL	-
Compound(s)	-	2 µL	-	-	-	-	2 µL	-	-	-
	discarded volume									
Control lysate	-	-	10 µL	-	-	-	-	10 µL	-	-
Supplemented Lysis buffer C (1x)	10 µL	10 µL	-	10 µL	10 µL	10 µL	10 µL	-	10 µL	10 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-	-	-	-	5 µL	-
Anti-H3-d2 Conjugate (methyl mark kit)	5 µL	5 µL	5µL	-	5 µL	-	-		-	-
Anti-Eu Cryptate Conjugate (methyl mark kit)	5 µL	5 µL	5 µL	5 µL	5 µL	-	-		-	-
Anti-H3-d2 Conjugate (total H3 kit)	-	-	-	-	-	5 µL	5 µL	5 µL	-	5 µL
Anti-H3-Eu3 Cryptate Conjugate (total H3 kit)	-	-	-	-	-	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL

* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

➥ *One-plate assay protocol in 20 µL final volume: standard protocol for suspension cells and adherent cells kept in medium for lysis*

	Specific methyl mark detection					Total Histone H3 detection				
	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	4 µL	4 µL	-	4 µL	-	4 µL	4 µL	-	4 µL	-
Cell culture Medium	2 µL	-	-	2 µL	6 µL	2 µL	-	-	2 µL	6 µL
Compound(s)	-	2 µL	-	-	-	-	2 µL	-	-	-
Control lysate	-	-	10 µL	-	-	-	-	10 µL	-	-
Supplemented Lysis buffer C (4x)	4 µL	4 µL	-	4 µL	4 µL	4 µL	4 µL	-	4 µL	4 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-	-	-	-	5 µL	-
Anti-H3-d2 Conjugate (methyl mark kit)	5 µL	5 µL	5 µL	-	5 µL	-	-	-	-	-
Anti-Eu Cryptate Conjugate (methyl mark kit)	5 µL	5 µL	5 µL	5 µL	5 µL	-	-	-	-	-
Anti-H3-d2 Conjugate (total H3 kit)	-	-	-	-		5 µL	5 µL	5 µL	-	-
Anti-H3-Eu3 Cryptate Conjugate (total H3 kit)	-	-	-	-	-	5 µL	5 µL	5 µL	5 µL	5 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL

* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

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