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Product information:

Document reference : 62KA2PAD rev01 (March 2014)

H3K4Me2 Cellular assay
10000 tests

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

Storage temperature: -60°C or below

Packaging details :

	384-well low volume plate (20 µL)
62KA2PAD	10000 tests

1. Assay description

This assay is intended for the simple, rapid and direct detection of endogenous levels of H3K4Me2 mark in cells. The dimethylation of Lysine 4 on histone H3 is detected in a sandwich assay format using 2 different specific antibodies, one labelled with Eu^{3+}-Cryptate (donor) and the second with d2 (acceptor).

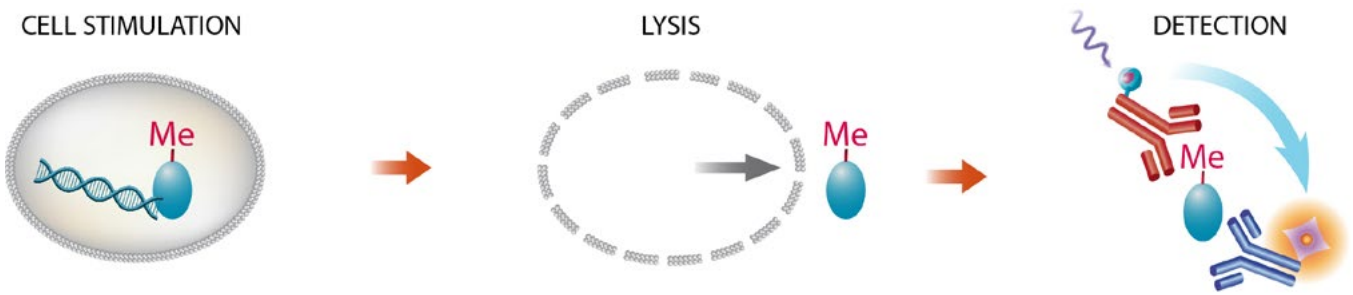
When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). One conjugate binds to Histone H3 and the other binds to H3K4Me2, thereby generating FRET. The specific signal modulates positively in proportion to dimethylation on Lysine 4.

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 H3K4Me2 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 H3K4Me2 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.

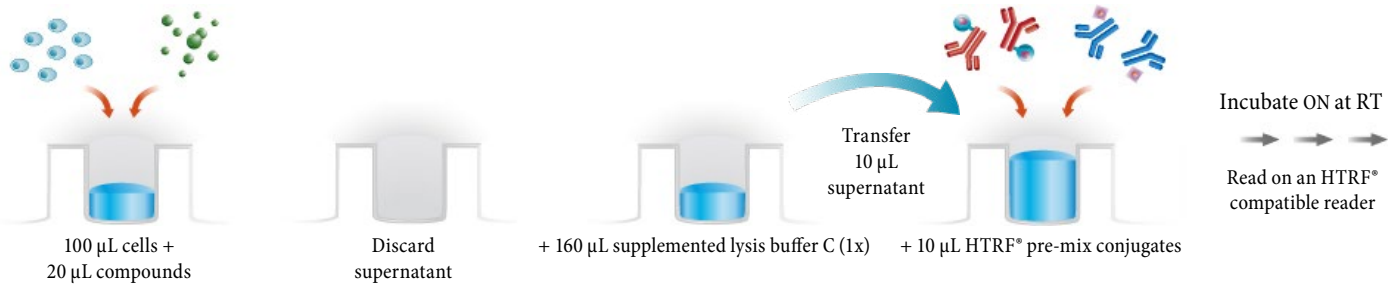
2. Companion product

Total H3 cellular assay (Cisbio Bioassays Ref # 62NH3PAE) can be used in parallel with H3K4Me2 cellular assays for normalization purpose for instance. In that case **refer to Appendix 1 of this document.**

3. Assay principle

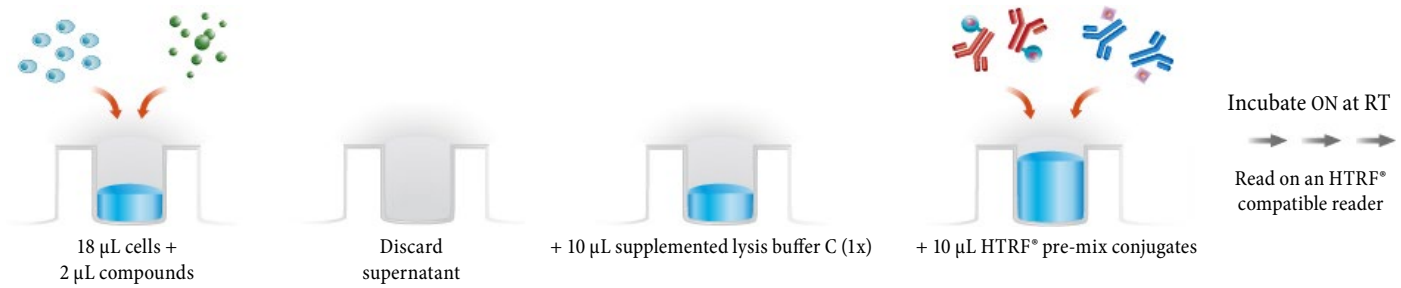


Two-plate assay protocol for adherent cells



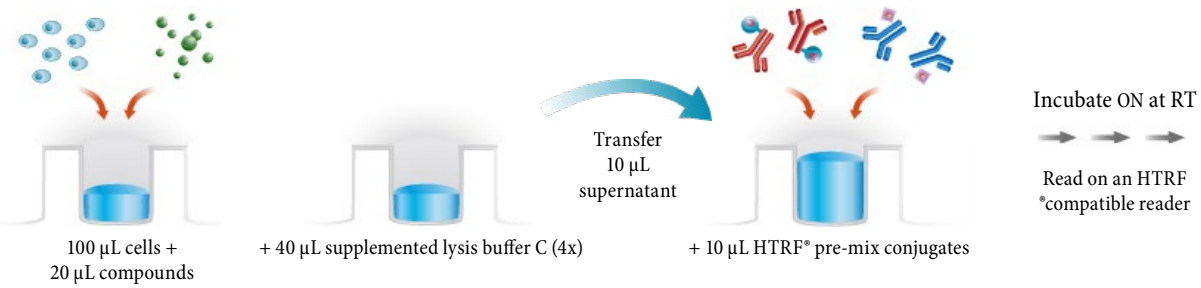
96-well plate **384-well plate SV**

One-plate assay protocol for adherent cells



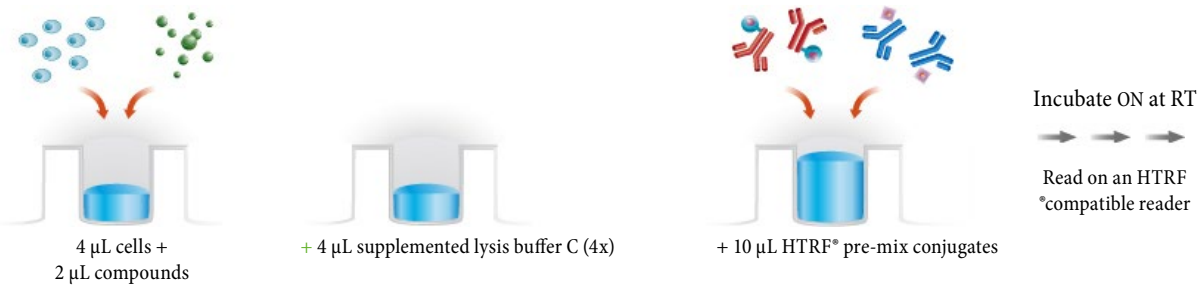
384-well plate SV

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



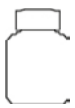
96-well plate **384-well plate SV**

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



384-well plate SV

4. HTRF reagents

		Nb of vials	Volume per vial	Storage	Ref # (when available separately)
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate (stock solution 50x)	 red cap	1	1 mL	-20°C or below	
Anti-H3-d2 Conjugate (H3K4Me2 kit) (stock solution 50x)	 blue cap	1	1 mL	-20°C or below	
H3K4Me2 Control Lysate (ready to use)	 green cap	3	150 µL	-60°C or below	62KA2TDA
EPIgeneous Lysis buffer C – Part 1 (stock solution 4x)	 Red cap	3	170 mL	-20°C or below	62EL3FDH
EPIgeneous Lysis buffer C – Part 2 (stock solution 100x)	 White cap	1	20 mL	-20°C or below	
Cellular Histone Detection Buffer (ready to use)	 White cap	1	120 mL	-20°C or below	

5. Storage stability

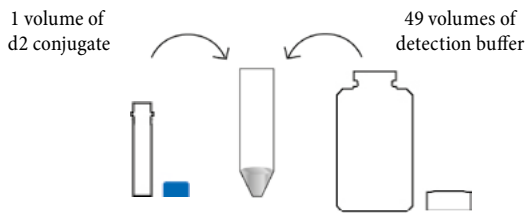
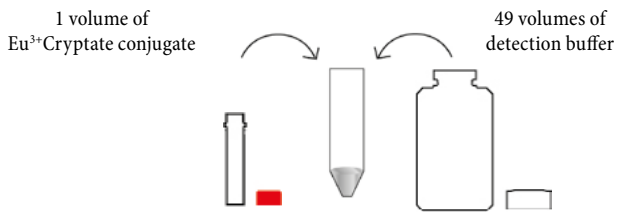
All reagents should be stored frozen until used.
To avoid freeze/thaw cycles, aliquot stock solutions into disposable plastic vials for storage at -20°C or below.
Thawed lysis and detection buffers can be stored at 2-8°C.

6. 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.

6.1. Preparation of conjugate working solutions

HTRF® reagent concentrations have been set for optimal assay performances.
Note that any dilution or improper use of the d2 and Eu^{3+}Cryptate-conjugates will impair the assay's quality.
Dilute the conjugates with detection buffer. In practice:

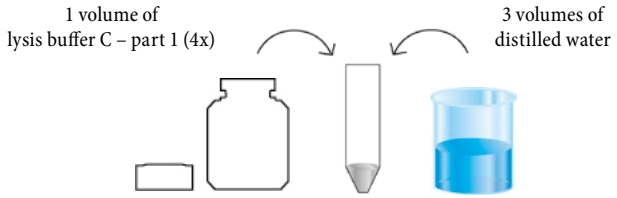
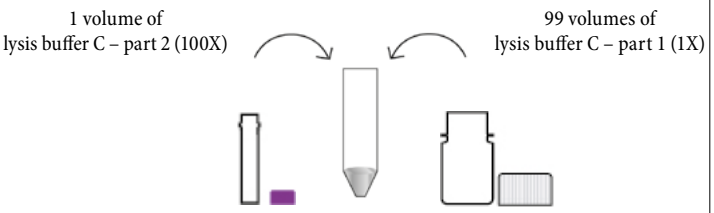
Anti-H3-d2 Conjugate (H3K4Me2 kit)	Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate
	
Dilute the frozen stock solution 50-fold with detection buffer: e.g. add 2450 µL of detection buffer to the 50 µL of conjugate stock solution (in different vial).	Dilute the frozen stock solution 50-fold with detection buffer: e.g. add 2450 µL of detection buffer to the 50 µL of conjugate stock solution. (in different vial).

6.2. Preparation of lysis buffer

Make sure to use the appropriate lysis buffer depending on the chosen protocol's specifications.
Make sure that lysate has been generated by using the kit reagents.

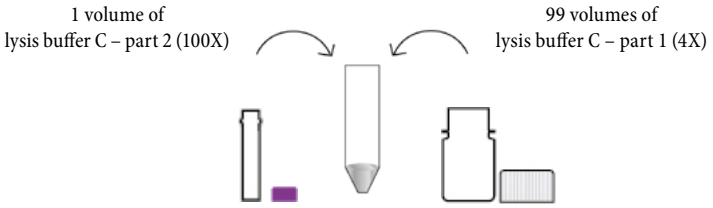
➥ *Supplemented lysis buffer C (1x) for two-plate & one-plate assay protocols for adherent cells*

Determine the amount of supplemented lysis buffer C (1x) needed for the experiment.
For the one-plate assay protocol, each well requires 10 µL of supplemented lysis buffer C (1x).
For the two-plate assay protocol, each well requires 160 µL of supplemented lysis buffer C (1x).
Prepare a lysis buffer C – Part 1 solution (1x) and then dilute the lysis buffer C – Part 2 (100x) 100-fold with this lysis buffer C – Part 1 (1x). In practice:

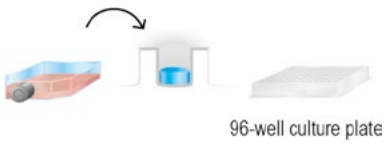
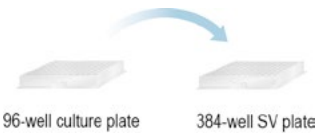
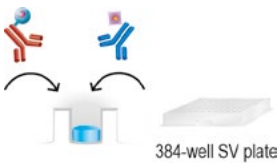
Preparation of lysis buffer C-part 1 (1x)	Preparation of supplemented lysis buffer C (1x)
	
Dilute the lysis buffer C - part 1 (4x) 4-fold with distilled water to prepare lysis buffer (1x). E.g. take 1.25 mL of lysis buffer (4x) and add it to 3.75 mL of distilled water. Mix gently.	Dilute the Lysis Buffer C – part 2 (100x) 100-fold with Lysis buffer C part 1 (1x). E.g. take 50 µL of Lysis Buffer C – part 2 and add it to 4.95 mL of Lysis buffer C part 1 (1x). Mix gently.

➥ *Supplemented lysis buffer C (4x) for two-plate and one-plate assay protocols for suspension cells and adherent cells kept in medium for lysis*

Determine the amount of supplemented lysis buffer C (4x) needed for the experiment.
For the two-plate assay protocol, each well requires 40 µL of supplemented lysis buffer C (4x).
Dilute the lysis buffer C – Part 2 (100x) 100-fold with this lysis buffer C – Part 1 (4x). In practice:

Preparation of supplemented lysis buffer C (4x)

Dilute the Lysis Buffer C – part 2 (100x) 100-fold with Lysis buffer C part 1 (4x). E.g. take 50 µL of Lysis Buffer C – part 2 and add it to 4.95 mL of Lysis buffer C part 1 (4x). Mix gently.

7. Two-plate assay protocol step by step

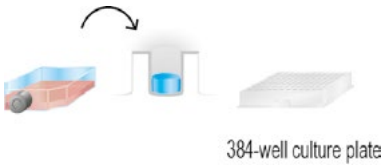
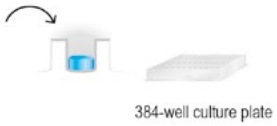
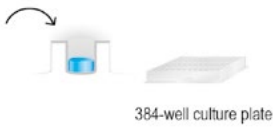
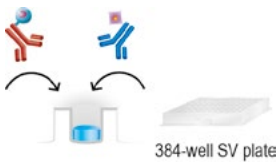
	For adherent cells	For suspension cells and adherent cells kept in medium for lysis	
1	Plate 100 µL of cells in a 96-well tissue-culture treated plate in appropriate growth medium. Incubate 3 to 4h at 37°C in CO_2 atmosphere in order to allow cell adhesion. Optimization of cell seeding densities is required.	Plate 100 µL of cells in a 96-well tissue-culture treated plate, in appropriate growth medium. Optimization of cell seeding densities is required. 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 C (1x) and incubate for 45 minutes at room temperature under shaking.	After desired incubation, add 40 µL of supplemented lysis buffer C (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 10µL 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.		
For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: www.cisbio.com/htrf-compatible-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	-	-	-
Supplemented lysis buffer C (1x) (depending on protocol used)	-	-	-	-	10 µL	-
Supplemented lysis buffer C (4x) (depending on protocol used)	-	-	-	-	-	4 µL
Growth medium	-	-	-	-	-	6 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-	-
Anti-H3-d2 Conjugate (H3K4Me2 kit)	5 µL	5 µL	5 µL	-	5 µL	5 µL
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate	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

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

8. 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.	
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 micro-titer plates for a long incubation (overnight and more). You absolutely must control this evaporation issue. 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 C (1x) and incubate for 45 minutes at room temperature. Use the appropriate lysis buffer.	Add 4 µL of supplemented lysis buffer C (4x) and incubate for 45 minutes at room temperature. Use the appropriate lysis buffer..	
5	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. 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/htrf-compatible-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	-	-
Supplemented lysis buffer C (1x)	10 µL	10 µL	-	10 µL	10 µL
Cellular Histone Detection Buffer	-	-	-	5 µL	-
Anti-H3-d2 Conjugate (H3K4Me2 kit)	5 µL	5 µL	5 µL	-	5 µL
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate	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.

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

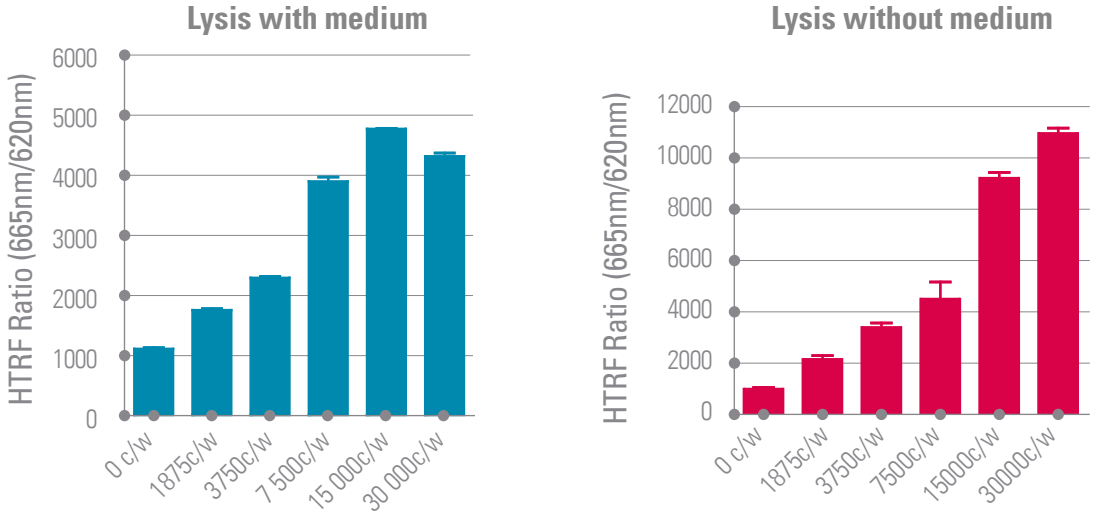
	Non stimulated cells	Stimulated cells	Control lysate	Blank control*	Negative control**
Cells	4 µL	4 µL	-	4 µL	-
Cell culture Medium	2 µL	-	-	2 µL	6µL
Compound(s)	-	2 µL	-	-	-
Control lysate	-	-	10 µL	-	-
Supplemented lysis buffer C (4x)	4 µL	4 µL	-	4 µL	4µL
Cellular Histone Detection Buffer	-	-		5 µL	-
Anti-H3-d2 Conjugate (H3K4Me2 kit)	5 µL	5 µL	5 µL	-	5 µL
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate	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. Data reduction

This data should only be considered as an example (readings on PHERAstarFS with flash lamp). Results may vary from one HTRF compatible reader to another. The histograms are drawn up by plotting HTRF Ratio versus number of cells seeded in 96-well plate.

Results on HeLa cells (various number of cells per well), using the two plate protocol for lysis with or without medium.
Cells were incubated 24h before lysis step according to the previously described protocol (Left panel, lysis with medium, right panel lysis wthout medium)



	Lysis with medium		Lysis without medium	
	HTRF Ratio (1)	CV% (2)	HTRF Ratio (1)	CV% (2)
0 c/w	1113	2%	1044	1%
1875c/w	1757	1%	2200	5%
3750c/w	2294	1%	3454	3%
7 500c/w	3898	2%	4562	13%
15 000c/w	4772	0%	9276	2%
30 000c/w	4315	1%	11030	1%

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/htrf-ratio-and-data-reduction		

10. How to improve your assay performances

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 in addition to special lids. (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 effects. 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 adjusted with lysis Buffer (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/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 (htrfservices@cisbio.com).

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INTERACTION IS EVERYTHING

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H3K4Me2 Cellular assay
10000 tests

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

Storage temperature: -60°C or below

Packaging details :

	384-well low volume plate (20 µL)
62KA2PAD	10000 tests

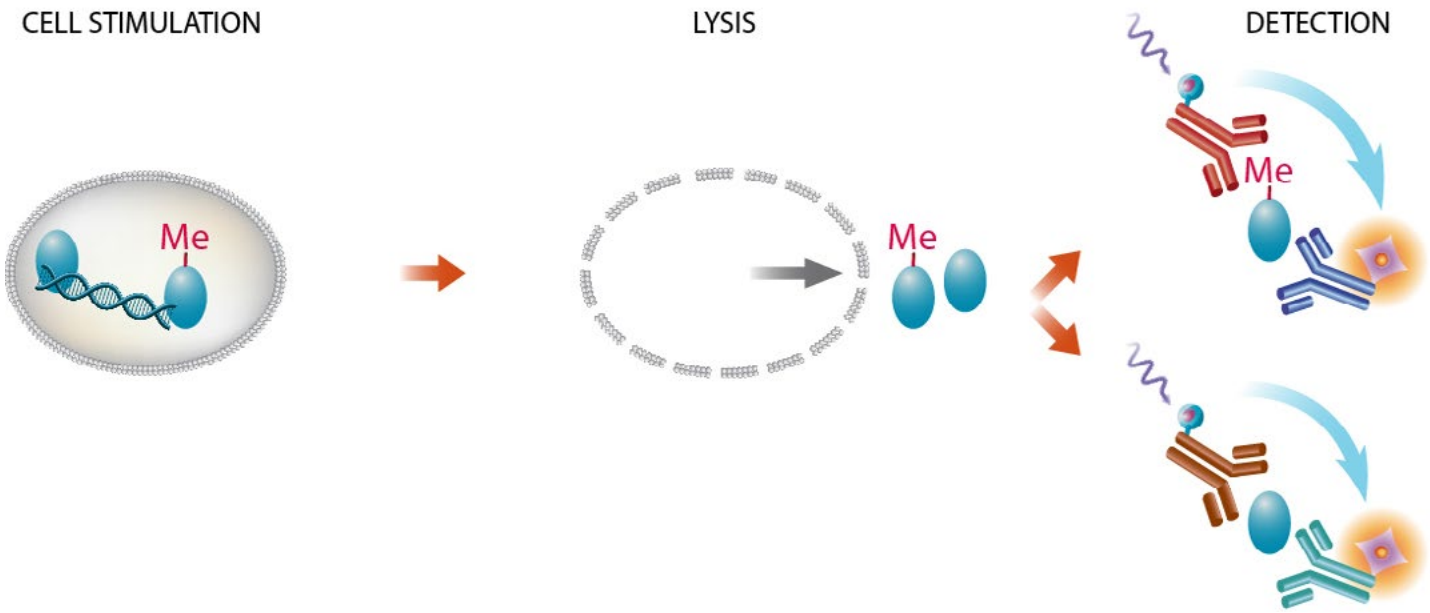
Product information:

Document reference : 62KA2PAD rev01 (Jan 2014)

Appendix 1: Assay protocols when using total H3 cellular assay in parallel

Total H3 cellular assay (#62NH3PAE) used in parallel with H3K4Me2 cellular assay

It is mandatory to use the lysis buffer included in this kit (H3K4Me2 cellular assay # 62KA2PAE) to generate the lysates that will be used for the detection of both the H3K4Me2 methyl mark and the total Histone H3



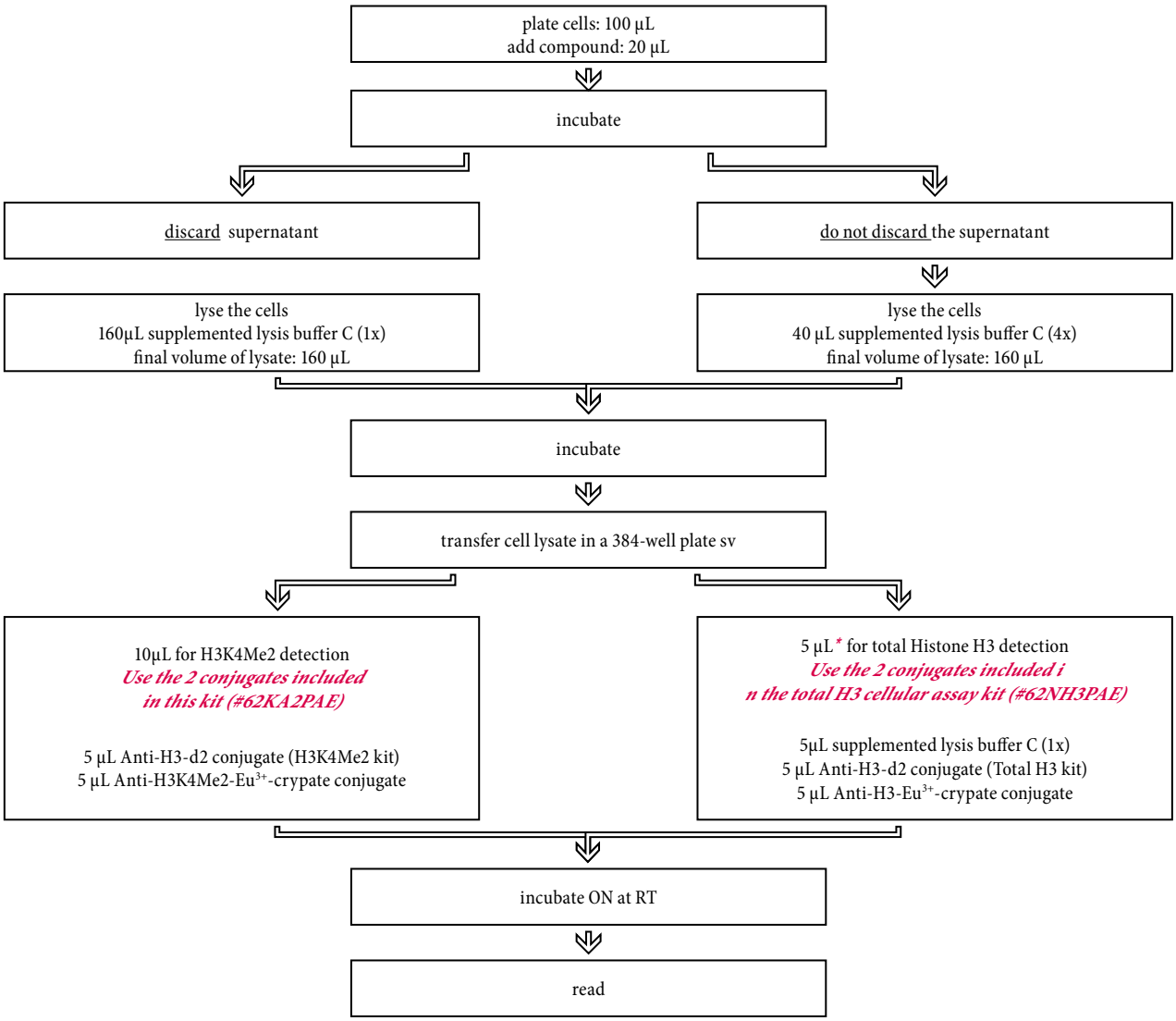
1. Two-plate assay protocols

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

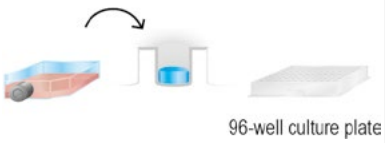
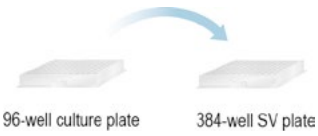
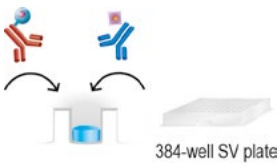
The volume of lysate generated in the two-plate assay protocols is sufficient for the detection of both the methyl mark and the total Histone H3

Use the lysis buffer included in the H3K4Me2 cellular assay to lyse the cells

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



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

	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 C (1x) and incubate for 45 minutes at room temperature under shaking.	After desired incubation, add 40 µL of supplemented lysis buffer C (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 10µL of this pre-mix is added. use the conjugates included in each kit: for H3K4Me2 detection (kit#62KD2PAE) 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/htrf-compatible-readers

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

	H3K4Me2 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 C (4x) (depending on protocol used)	-	-	-	-	4 µL	-	-	-	-	-	4 µL	-
Supplemented lysis C (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 (H3K4Me2 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	5 µL
Anti-H3K4Me2-Eu^{3+}Cryptate Conjugate	5 µL	5 µL	5 µL	5 µL	5 µL	5 µL	-	-	-	-	-	-
Anti-H3-Eu^{3+} 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

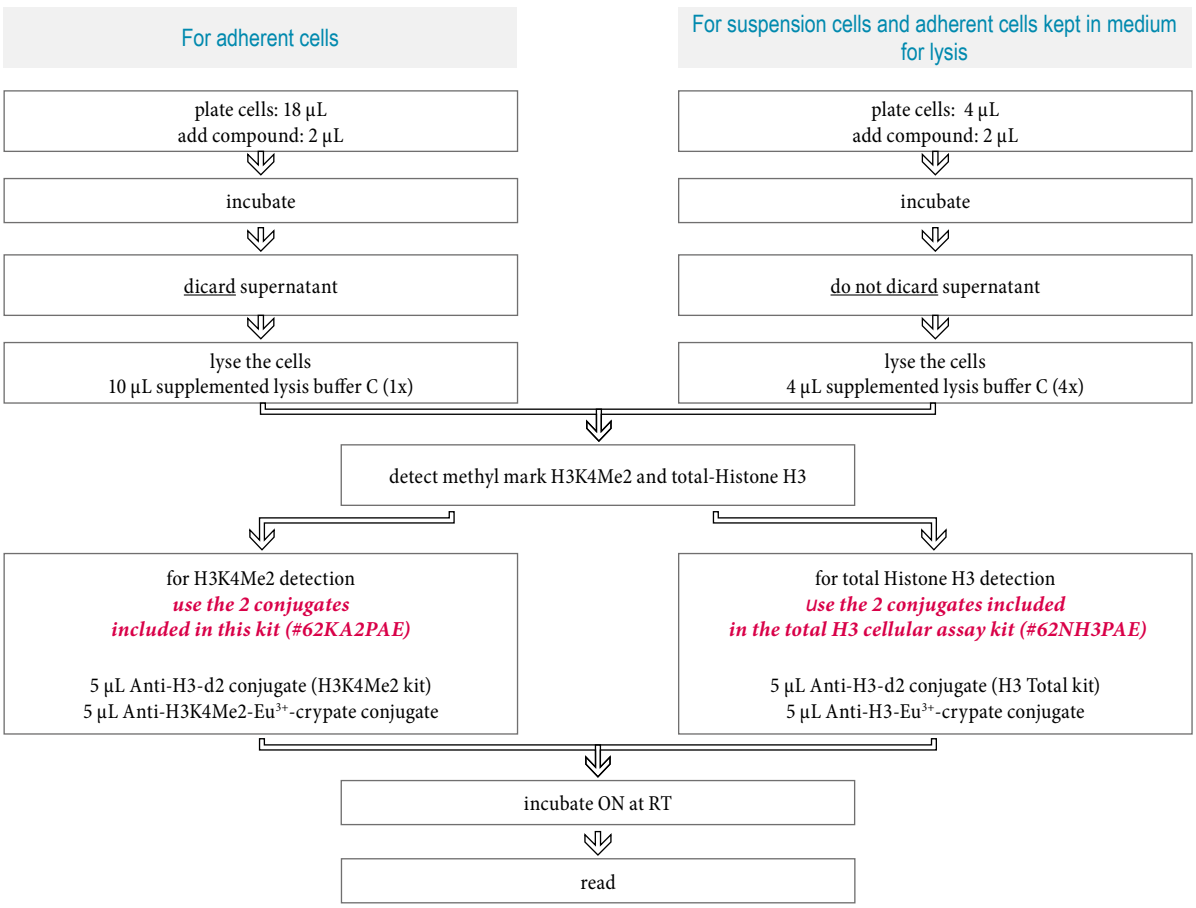
* Blank control is used to check the Cryptate signal at 620 nm. ** Negative control is used to check the Non specific signal.

2. One-plate assay protocols

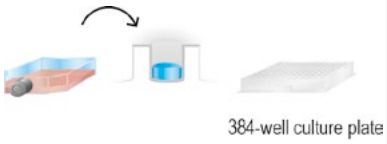
As cell seeding, cell stimulation, cell lysis and cell detection are performed in a single well, the 2 assays (H3K4Me2 and total Histone H3) have to be performed in parallel.

Use the lysis buffer included in the H3K4Me2 cellular assay to lyse the cells

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 C (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 C (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® conjugate, prepared in the detection buffer. The 2 conjugates can also be pre-mixed JUST PRIOR to dispensing, and 10µL of this pre-mix is added. Use the conjugates included in each kit: for H3K4Me2 detection (kit#62KA2PAE) 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.		

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

	H3K4Me2 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 (H3K4Me2 kit)	5 µL	5 µL	5µL	-	5 µL	-	-		-	-
Anti-H3-d2 Conjugate (total H3 kit)	-	-	-	-	-	5 µL	5 µL	5 µL	-	5 µL
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate	5 µL	5 µL	5 µL	5 µL	5 µL	-	-		-	-
Anti-H3-Eu^{3+} 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*

	H3K4Me2 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 (H3K4Me2 kit)	5 µL	5 µL	5 µL	-	5 µL	-	-	-	-	-
Anti-H3-d2 Conjugate (total H3 kit)	-	-	-	-		5 µL	5 µL	5 µL	-	-
Anti-H3K4Me2-Eu^{3+} Cryptate Conjugate	5 µL	5 µL	5 µL	5 µL	5 µL	-	-	-	-	-
Anti-H3-Eu^{3+} 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.