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Product information:

Document reference : 63ADK088PEH rev 01 - Aug-17

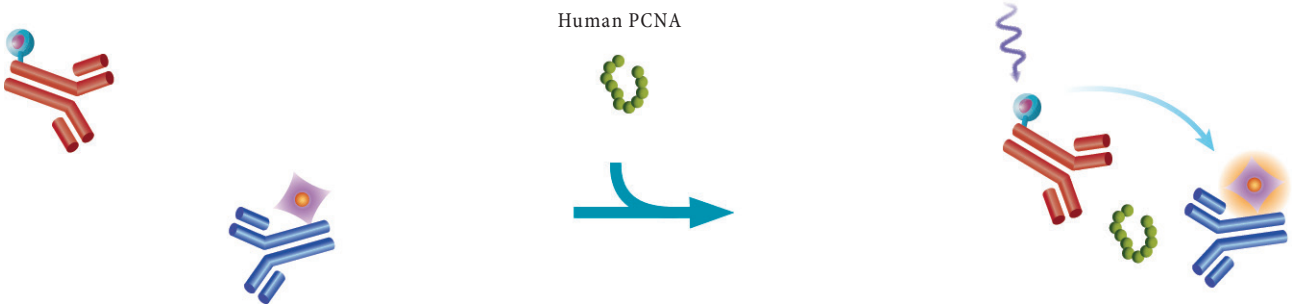
Human PCNA kit
10,000 tests

For in vitro research use only

Storage temperature: -60°C or below

1. ASSAY DESCRIPTION

This assay is intended for the quantitative measurement of Human PCNA in cells.



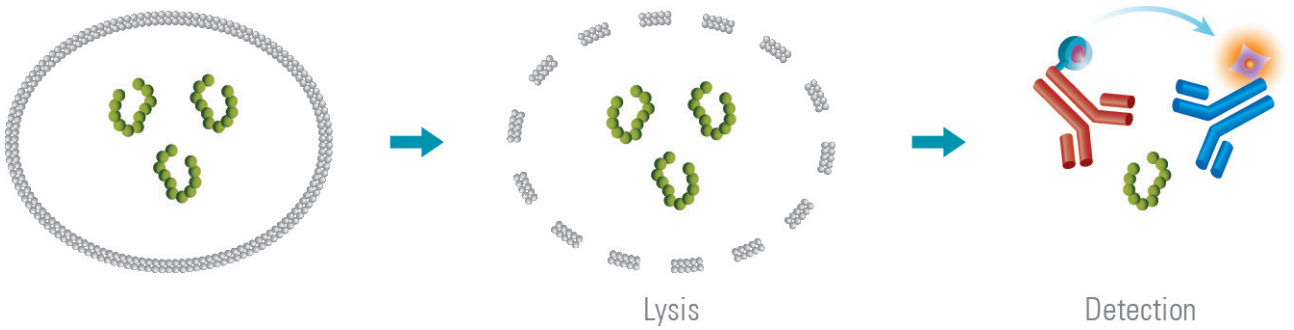
As shown here, Human PCNA is detected in a sandwich assay format using 2 different specific antibodies, one labelled with Tb3+-Cryptate (donor) and the second with d2 (acceptor).

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 Human PCNA.

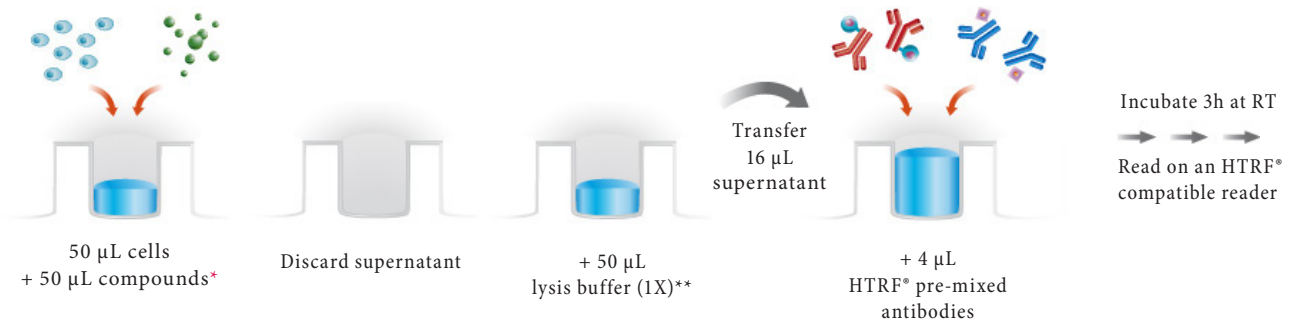
The assay is run under a two-plate assay protocol, where cells are plated, stimulated and lysed in the same culture plate. Lysates are then transferred to the assay plate for the detection of Human PCNA by HTRF® reagents. This protocol gives the ability to monitor the cell viability and confluence.

Technical support team can help you to set-up this protocol. Please contact us at http://www.cisbio.com/drug-discovery/contact-us.

2. PROTOCOL AT A GLANCE



➥ TWO-PLATE ASSAY PROTOCOL (FOR ADHERENT CELLS)



* Note that concentration above 0.5% DMSO will impair assay performance.

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

96-well plate → **384-well white plate SV**

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3. HTRF® REAGENTS

Human PCNA Cryptate antibody	red cap	1	0.4 mL	-20°C or below	
Human PCNA d2 antibody	blue cap	1	0.4 mL	-20°C or below	
Control lysate (ready-to-use)	green cap	2	150 µL	-60°C or below	63ADK088TDA
Lysis buffer* (stock solution 4X)	white cap	1	130 mL	-20°C or below	64LB1FDF
detection buffer (ready-to-use)	red cap	1	50 mL	-20°C or below	

* Amounts of reagents provided are sufficient for generating 50 µL of cell lysate per well. For higher volumes, extra vials can be ordered separately.

4. STORAGE STABILITY

Antibodies, control lysate and buffers should be stored frozen until use.
Thawed lysis buffer, detection buffer and blocking reagent can be stored at 2-8°C in your premises.
Thawed antibodies are stable 48 hours at 2-8°C; they can be refrozen (at -20°C or below) and thawed at least one more time.
Control lysate must be stored frozen at -60°C or below. Thawed control lysate can be refrozen (at -60°C or below) and thawed one more time.

5. 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.

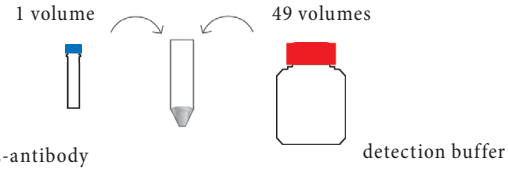
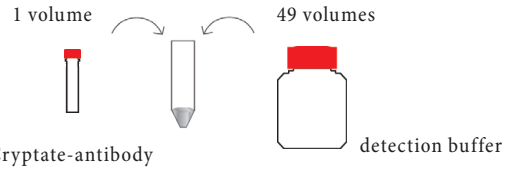
5.1. Control lysate solution: ready-to-use

The control cell 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.

5.2. Preparation of antibody working solutions

HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the d2 and Cryptate-antibodies will impair the assay's quality. Be careful, as working solution preparation for antibodies may differ between the 500 and 10,000 data point kit.

Antibody working solutions are stable for 2 days at 4°C. Dilute the antibodies with detection buffer . In practice:

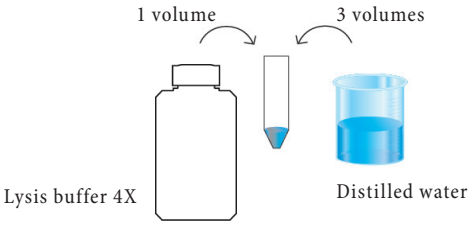
Human PCNA d2 antibody	Human PCNA Cryptate antibody
	
Dilute 50-fold the frozen stock solution with detection buffer : e.g. add 49 mL of detection buffer to the 1 mL of d2-antibody stock solution.	Dilute 50-fold the frozen stock solution with detection buffer : e.g. add 49 mL of detection buffer to the 1 mL of Cryptate-antibody stock solution.

5.3. Preparation of lysis buffer

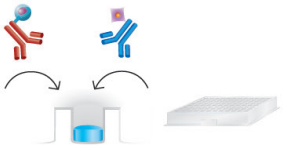
Make sure that the lysate has been generated by using the kit reagents.

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
Determine the amount of lysis buffer needed for the experiment. Each well requires generally 50 µL of lysis buffer.
Prepare a lysis buffer solution 1X by diluting 4-fold the lysis buffer 4X with distilled water. In practice:

Preparation of lysis buffer 1X

Dilute the "lysis buffer 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.

6. TWO-PLATE ASSAY PROTOCOL

	For adherent 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. Cell seeding densities of 100 K 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 necessary.	 96-well culture plate
2	Dispense 50 µL of compounds (2X) diluted in cell culture medium For most compounds, incubation time should be above 24 hours at 37°C. We recommend a time course study to determine the optimal stimulation time. Note that concentration above 0.5% DMSO will impair assay performances. Same final concentration of DMSO must be used for each compound dilutions.	 96-well culture plate
3	Remove carefully cell supernatant either by aspirating supernatant or by flicking the plate.	Discard supernatant (for adherent cells)  96-well culture plate
4	Immediately add 50 µL of lysis buffer (1X) and incubate for at least 30 minutes at room temperature under shaking. Use the appropriate lysis buffer and incubate at room temperature with shaking. We recommend a time course study to determine the optimal lysis incubation time. Lysis volume can be decreased down to 25 µL.	 96-well culture plate
5	After homogenization by pipeting up and down, transfer 16 µL of cell lysate from the 96-well cell-culture plate to a 384-well small volume white 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 384-well SV plate
6	Add 4 µL of premixed antibody solutions (vol/vol) prepared in the detection buffer . Cover the plate with a plate sealer. Incubate 3h at room temperature. Set up your reader for Tb3+ Cryptate and read the fluorescence emission at two different wavelenghts (665nm and 620nm) on a compatible HTRF® reader**.	 384-well SV plate
**For more information about HTRF® compatible readers and for set-up recommendations, please visit our website at: http://www.cisbio.com/readers		

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

	Non treated cell lysate	Treated cell lysate	Control lysate	Blank control	Negative control
Non treated cell lysate	16 µL	-	-	16 µL	-
Treated cell lysate	-	16 µL	-	-	-
Control lysate	-	-	16 µL	-	-
Lysis buffer (1X)	-	-	-	-	16 µL
detection buffer	-	-	-	2 µL	-
Human PCNA d2 antibody	2 µL	2 µL	2 µL	-	2 µL
Human PCNA Cryptate antibody	2 µL	2 µL	2 µL	2 µL	2 µL
Total volume	20 µL	20 µL	20 µL	20 µL	20 µL

The blank control is used to check the Cryptate signal at 620 nm.

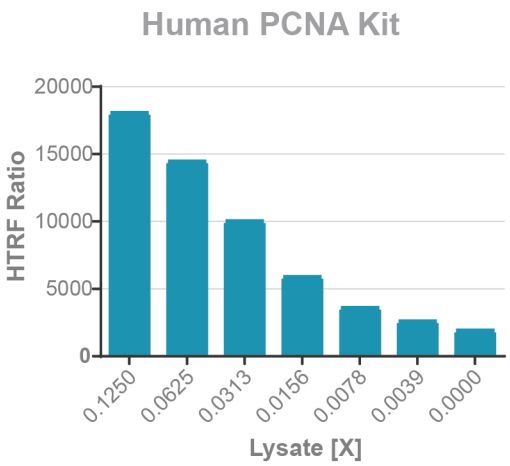
The Negative control is used to check the non-specific signal. The ratio between control lysate signal / non-specific signal should be greater than 2.

7. DATA REDUCTION

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

PHERAstarFS with flash lamp (BMG) was used for reading.

	Human PCNA	
Lysates [X]	ratio(1)	CV% (2)
0.125	18083	1.6%
0.0625	14469	2.2%
0.0313	10040	0.8%
0.0156	5913	1.8%
0.0078	3611	1.4%
0.0039	2605	1.2%
no cells	1922	4.6%



Ratio	Signal 665nm ---------- x 104 Signal 620nm	Ratio must be calculated for each individual well.
CV%	Standard deviation ---------- x 100 Mean ratio	The mean and standard deviation can then be worked out from ratio replicates.
For more information about data reduction, please visit our website at: http://www.cisbio.com/data-reduction		

Technical support team can help you to set-up this protocol, please contact us at http://www.cisbio.com/drug-discovery/contact-us.