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Product information:

Document reference : 64HISPEG

Rev 01-January 2019

6His check kit Gold 500 tests

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

Storage temperature: -60°C or below

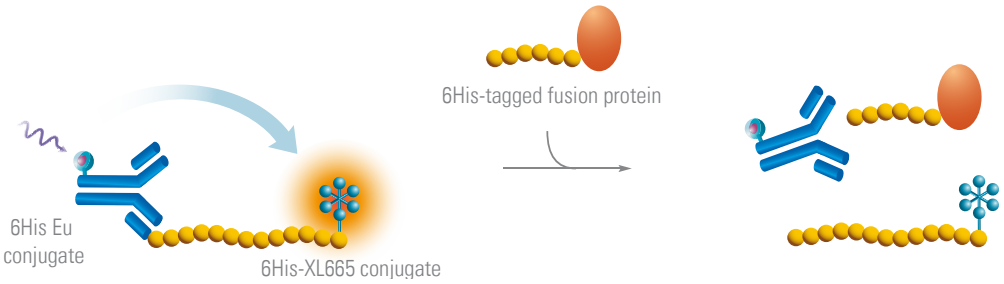
Packaging details :

384-well low volume plate (20 µL)

64HISPEG	500 tests

1. ASSAY DESCRIPTION AND INTENDED USE

The 6His check kit Gold enables the rapid detection of 6His tagged fusion proteins using the HTRF® technology. It can either be used to ensure that the 6His motif of a fusion protein is accessible to the anti-6His antibody, or to approximate the concentration of a 6His-tagged protein.

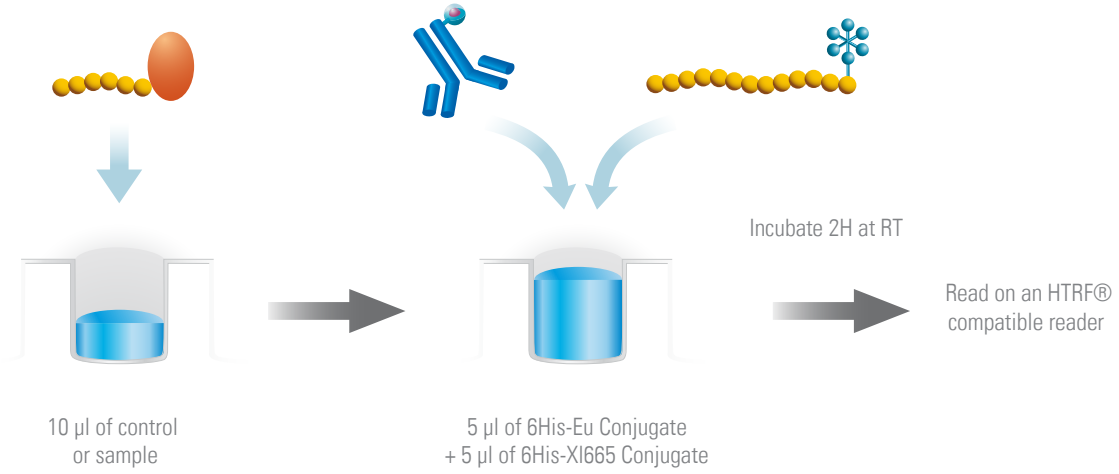


As shown in the graph, 6His peptide labeled with XL665 is detected by anti 6His Cryptate conjugate.

The 6His-tagged fusion protein to be ascertained competes with the 6His-peptide-XL665 conjugate for binding to the anti-6His-Cryptate conjugate.

The specific signal is inversely proportional to the concentration of 6His-tagged fusion protein.

2. PROTOCOL AT A GLANCE



Caution! Make sure to add control or sample before conjugates.

3. SUPPLIED REAGENTS AND STABILITY

3.1. Supplied reagent s

		Number of vials	Volume per vial	Storage
6His check kit Gold Eu conj	 orange cap	1	50 µL	-20°C or below
6His check kit Gold XL665 conj	 blue cap	1	50 µL	-60°C or below
6His check kit Gold control	 green cap	1	100 µL	-20°C or below
Diluent #7– 20 mL	 white cap	1	20 mL	-20°C or below
Detection buffer #7 – 13 mL	 red cap	1	13 mL	-20°C or below

3.2. Storage & stability

➥ Conjugates and control: should be stored frozen until use. To avoid freeze/thaw cycles, it is recommended to dispense all remaining stock solutions of conjugates into disposable plastic vials for storage at -60°C or below. After thawing, they can be refrozen and thawed once.

➥ Buffers: Thawed buffers can be stored at 2-8°C in your premises before opening. Opened buffers are stable 48 hours at 2-8°C. All buffers should be stored at -20°C or below for long term storage.

To avoid freezing and thawing cycles, it is recommended to aliquot and freeze all the reagents.

4. REAGENT PREPARATION

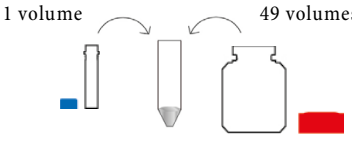
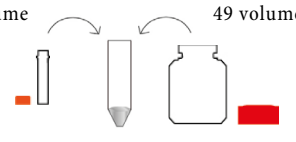
HTRF® reagent concentrations have been set for optimal assay performances. Note that any dilution or improper use of the XL665 and Cryptate-conjugates will impair the assay's quality.
For an accurate quantitative determination of sample, dilution of your standard must be carried out with the medium used for preparing the samples (i.e. diluent #7 or other diluent, culture medium or any other compatible medium).
Thaw all reagents at room temperature, allow them to warm up. Centrifuge shortly the vials to be sure to pipet all the required volume. Prepare the working solutions from stock solutions (§3) by following the instructions below.

4.1. Preparation of buffers

Diluent #7 and detection buffer # 7 are ready to use solutions.

4.2. Preparation of conjugate working solutions

Determine the amount of conjugate needed for the experiment. Each well requires 5 µL of each conjugate.

 6His-XL665 conjugate Reconstitution buffer #7	 6His-Eu Cryptate-Conjugate Reconstitution buffer #7
Dilute 50-fold the thawed stock solution with detection buffer#7 : e.g. add 2.45 mL of detection buffer #7 to 0.05 mL of conjugate stock solution	Dilute 50-fold the thawed stock solution with detection buffer #7: e.g. add 2.45 mL of detection buffer #7 to 0.05 mL of conjugate stock solution

4.3. Preparation of the 6His kit control

The control is ready to use. Determine how many replicates are to be tested. Each well requires 10 µl of control.

5. ASSAY PROTOCOL

Dispense the reagents in the following order:

Step 1
10 µL of control or sample

Step 1
5 µL of 6His-Xl665 conjugate

Step 1
5 µL of 6His-Eu conjugate

Cover the plate with a plate sealer. Incubate 2H at RT.

Remove the plate sealer and read the fluorescence emission at two different wavelengths (665nm and 620nm) on an HTRF® compatible reader.

For more information about HTRF® compatible readers, please visit our website at: http://www.cisbio.com/htrf-compatible-readers

	Assay controls			
	Buffer control	Negative & Cryptate control	Positive control	Sample or 6His kit control
	Used to check the background fluorescence	Used to check the cryptate signal at 620 nm at to calculate the %DF or the assay window	Used to obtain the maximum signal	
Sample or 6His kit control	-	-	-	10 µL
Diluent #7	10 µL	10 µL	10 µL	-
6His check kit Gold XL665 conj	-	-	5 µL	5 µL
6His check kit Gold Eu conj	-	5 µL	5 µL	5 µL
Detection buffer # 7	10 µL	5 µL		-

6. ASSAY FLEXIBILITY AND MINIATURIZATION

When used as recommended, the kit will provide sufficient reagents for 500 tests using a 384- well low volume plate in 20 µL final assay volume.

To change to other plate formats (96 half-well or 1536-well) and final volumes (100 µL to less than 10 µL), the volume of each assay component is simply proportionally adjusted in order to keep the reagent concentrations the same as for the 20 µL final assay volume. For instance, in the case of the 1536-well format in 10 µL final volume, half as much material per well is used, thereby enabling 2,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

Assay format				
Assay components	Volume proportion	1536-well (10 µl)	384-well low volume (20 µl)	96 half-well (100 µl)
6His control or sample	2 volumes	5 µl	10 µl	50 µl
Eu conjugate	1 volume	2.5 µl	5 µl	25 µl
XL665 conjugate	1 volume	2.5 µl	5 µl	25 µl
	Bulk size	1,000 tests	500 tests	100 tests

White Plate references: 96 half-well plate (Costar # 3693 or equivalent), 384-well low volume plate (Greiner # 784075), 1536-well (Greiner # 782080).

7. DATA REDUCTION

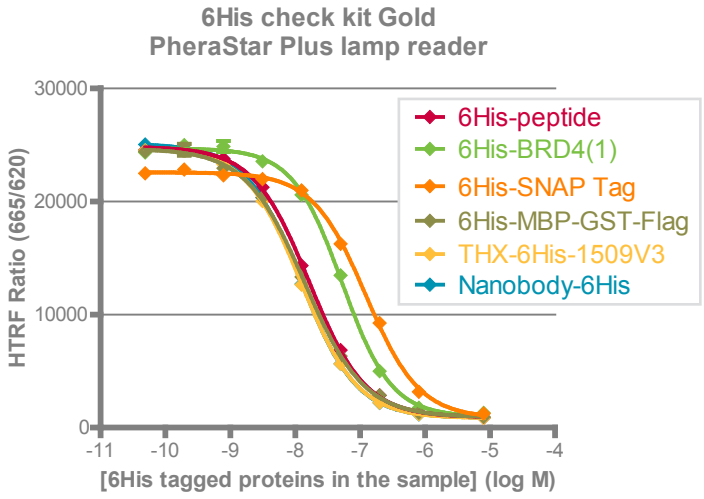
	Ratio (1)	CV% (2)	Delta F% (3)	% inhibition
Negative control	810	0.9 %		
Positive control	29526	0.7 %	3546%	0%
6His kit control	15381	1.2 %	1799%	49.5 %

The 6His kit Control should give approximately between 40% and 60% of signal inhibition.

Ratio (1)	$\frac{\text{Signal}_{665nm}}{\text{Signal}_{620nm}} \times 10^4$	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.
Delta F% (3)	$\frac{\text{Ratio}_{\text{6His control or sample}} - \text{Ratio}_{\text{Negative control}}}{\text{Ratio}_{\text{Negative control}}} \times 100$	Reflects the signal to background of the assay. The negative control plays the role of an internal assay control.
For more information about data reduction, please visit our website at: www.cisbio.com/htrf-ratio-and-data-reduction		

To obtain additional information or support, please contact your technical support team (htrfservices@cisbio.com).

8. CALIBRATION CURVE



Tag insertion	6His Tagged protein	MW (Da)	IC50 (nM)
N-term	6His-peptide (7AA)	1,3	32.8
	6His BRD4(1)	17,8	105.0
	6His-SNAP	20,6	244.8
	6His-MBP-GST-Flag	64	28.0
Internal	THX-6His-1509V3	27	24.8
C-term	6His tagged Nanobody	15	24.4

6His check kit Gold enables the verification of the tag accessibility on 6His-tagged fusion proteins. As shown in the figure, the recognition of the 6His tag is highly dependent on the tag position, and the construct. Quantitative determination of a given fusion protein should only be carried out on a relative basis and implies the introduction of an internal calibrator (i.e. a closely related 6His fusion protein of known concentration) which can be used as a reference material.