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6HIS-tag check kit

For in vitro research use only

Storage temperature : 2-8°C

www.htrf.com

HTRF® package insert

Document reference : 62HISPEB rev04 (July 2008)

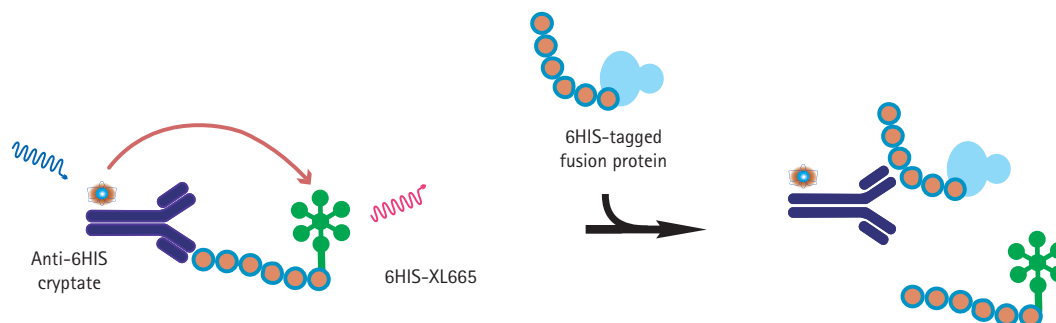
Packaging details :

	384-well low volume plate (20 µL)
62HISPEB	1,000 tests

1. Assay description and intended use

This kit enables the rapid detection of 6HIS-tagged fusion proteins. 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.

Its principle is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown below, 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 the binding to the anti-6HIS-Cryptate conjugate. Specific signal (i.e. energy transfer) is inversely proportional to the concentration of 6HIS-tagged fusion protein.

2. Background

The development of fusion protein technology has boosted the use of toolbox reagents for the purification and the detection of recombinant proteins. This technique consists of the addition of a specific sequence (i.e. tag) to the protein to be expressed. These tags can be inserted at different places in the sequence and are often added to N or C-terminal ends to guarantee the production of a biologically active recombinant protein. The protein can then be detected through the tag using toolbox reagents (e.g. antibodies raised against this tag or proteins having an affinity for it).

6HIS tag – six consecutive histidine amino acid residues – is a tag widely used in molecular biology. Both eukaryotic and bacterial expression vectors for the production of 6HIS tagged proteins are commercially available. In most cases the 6HIS tag does not interfere with the structure or the function of the recombinant protein (enzymes,...). 6HIS motif also allows the purification of the protein on metal chelating solid phases.

3. Protocol

3.1. Supplied reagents and reconstitution

Supplied reagents	Reagent reconstitution (stock solutions)	Working solutions
Anti-6HIS-Cryptate**	1 vial Lyophilized*	Add 5 mL of reconstitution buffer to each vial. Mix gently.
6HIS peptide-XL665	1 vial Lyophilized*	
6HIS assay control Concentrated 6HIS peptide	1 vial Lyophilized*	See indication on label for reconstitution volume. Mix gently.

Reconstitution buffer 50 mM Phosphate buffer, pH 7.0, 0.8M KF	1 vial See volume on the label
Diluent 50 mM Phosphate buffer, pH 7.0, 0.2% BSA, preservatives, NaN ₃	1 vial of 20 mL

* All reagents were lyophilized in 50 mM phosphate buffer, pH 7, containing BSA protease free and stabilizers.

** The Cryptate conjugate concentration was optimized for a maximum assay sensitivity and to ensure an average counting of 40,000 cps at 620 nm (384-well low volume plate), using the reference PHERAstar Plus reader (BMG LABTECH).

Allow the reagents to warm up at room temperature for at least 30 mins and reconstitute all vials as indicated above.

Precaution : 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.

3.2. Reagent stability

All reagents should be stored at 2-8°C until reconstituted. It is better to reconstitute the two conjugates just before dispensing them. Once reconstituted, the reagents may be frozen once and stored at -20°C for no longer than 2 months. Avoid repeated freezing and thawing.

3.3. Sample preparation

Dilute all samples to be assayed with the diluent. Consecutive dilutions should be made within the 2 to 500 nM range (working solution).

3.4. Assay protocol for 384-well low volume plate (20 µL)

⇒ Dispense the reagents in the following order :

- 10 µL control or sample *
- 5 µL Anti-6HIS-Cryptate
- 5 µL 6HIS peptide-XL665

* For negative control, replace the control by 10 µL of diluent and the 6HIS peptide-XL665 by 5 µL of reconstitution buffer

* For positive control, replace the first reagent by 10 µL of diluent

⇒ Cover the plate with a plate sealer and leave to incubate at 2-8°C for 2 hours.

⇒ Read on a compatible HTRF® reader (more information about compatible reader at htrf-assays.com/readers).

3.5. Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 1,000 tests using using a 384- well low volume plate in 20 µL final assay volume (HTRF® packaged basis).

To move 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 maintain the reagent concentrations 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 allowing 2,000 tests to be run. The performances of the HTRF® assay remain the same whatever the level of miniaturization.

Assay components	Volume proportion	Assay format		
		1536-well (10 µL)	384-well low volume (20 µL)	96 half-well (100 µL)
Sample	2 volumes	5 µL	10 µL	50 µL
XL665 conjugate	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
		2,000 tests	1,000 tests	200 tests

Plate references : 96 half-well plate (Costar # 3694 or equivalent), 384-well low-volume plate (Greiner # 784076), 1536-well (Greiner # 782086)

3.6. Data reduction

Results are expressed in Delta F

$$1. \text{Ratio} = \frac{A_{665\text{nm}}}{B_{620\text{nm}}} \times 10^4$$

$$2. \text{Mean Ratio} = \frac{\sum \text{ratios}}{2}$$

$$3. \text{CV} = \frac{\text{Std deviation}}{\text{Mean ratio}} \times 100$$

$$4. \text{Delta F} = \frac{\text{Calibrator or sample Ratio} - \text{Ratio}_{\text{neg}}}{\text{Ratio}_{\text{neg}}} \times 100$$

(Ratio_{neg} = negative control)

	A (665nm)	B (620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)	Delta F / Delta F _{max} %	% of inhibition
Negative control	2032 1959	45252 43516	449 450	450	0.2			
0 Positive control	24143 28117	35620 42627	6778 6596	6687	1.9	1387	100	0
6HIS control	14814 14461	43161 43251	3432 3344	3388	1.9	654	47	53

Based on these calculations, the kit control should give approximately between 40 and 60% of signal inhibition.

6HIS check kit enables the verification of the tag accessibility on 6HIS-tagged fusion proteins. As shown below, the recognition of the 6HIS tag is highly dependent on the tag position and the number of motifs in the construct, as well as on the buffer type. 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.

