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FLAG check kit
10,000 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)
63ADK036PEH	10,000 tests

Product information:

Document reference : 63ADK036PEH - Rev 02-Sept.2019

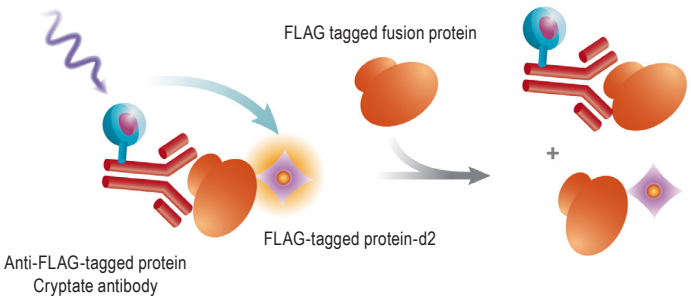
1. ASSAY DESCRIPTION AND INTENDED USE

This assay is intended for the quantitative determination of FLAG-tagged protein using HTRF® technology.

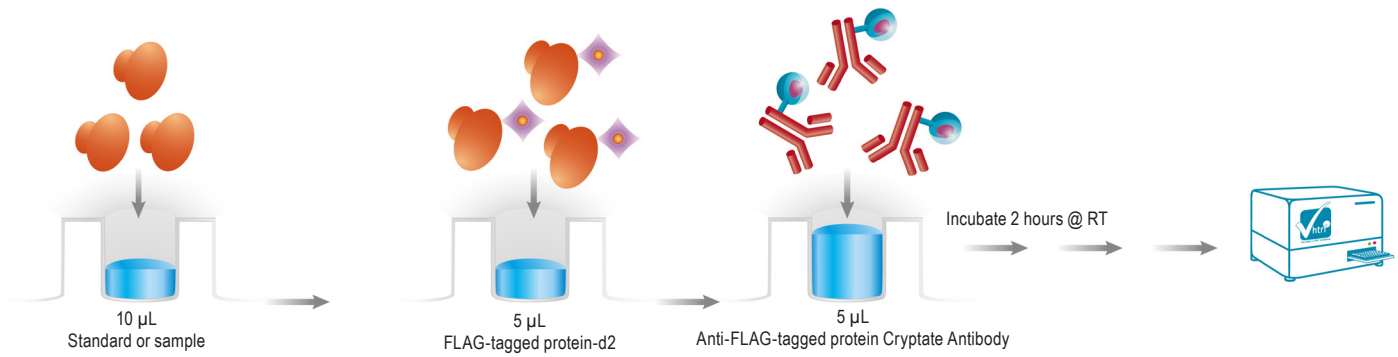
As shown in the diagram to the right, FLAG-tagged protein (FLAG) is detected in a competitive assay format using a specific antibody labelled with Eu^{3+}-Cryptate (donor) and FLAG-tagged peptide labelled with d2 (acceptor).

When these 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 (665nm).

The FLAG-tagged protein present in the sample competes with the binding between the two conjugates and thereby prevents FRET from occurring. The specific signal, inversely proportional to FLAG-tagged protein concentration, modulates negatively.



2. PROTOCOL AT GLANCE



3. HTRF REAGENTS

	FLAG-tagged standard	FLAG-tagged peptide d2	Anti-FLAG Eu^{3+}-Cryptate-antibody	Diluent	Detection buffer # 3
					
Stock solution	10 µL/vial 10 µM	1 mL/vial	1 mL/vial	20 mL/vial	105 mL/vial
Storage	-60°C or below	-60°C or below	-60°C or below	4°C to -60°C*	4°C to -60°C*
Ref# (when available sperately)	-	-	-	62DL1DDD	-

* Diluent and Detection buffer are shipped frozen, but can be stored at 2-8°C.

4. REAGENT PREPARATION

HTRF® reagent concentrations have been set for optimal assay performance. Any dilution or improper use of the d2 and Cryptate-antibodies will impair the quality of the assay.

For an accurate quantitative determination of sample, dilution must be carried out with the medium used for preparing the samples (i.e. diluent, culture medium or any other compatible medium).

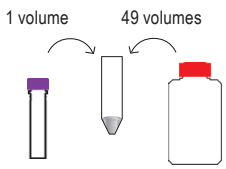
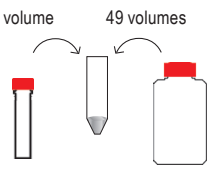
Antibodies may be frozen and thawed once: to avoid freeze/thaw cycles it is recommended that you dispense remaining stock solutions of standard and antibodies into disposable plastic vials for storage at –60°C or below.

Please note, working solution preparation may differ between the 500 and the 10,000 test size kits.

- Thaw all reagents at room temperature, allow them to warm up.
- Prepare the working solutions from stock solutions by following the instructions below.

4.1. Preparation of antibody working solutions

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

FLAG-tagged peptide-d2	Anti-FLAG-Eu^{3+}-Cryptate-antibody
	
Dilute 50-fold the stock solution of FLAG-tagged protein-d2 with detection buffer: e.g. take 1 mL of d2 stock solution and add it to 49 mL of detection buffer # 3.	Dilute 50-fold the stock solution of anti-human FLAG-tagged protein-cryptate antibody with detection buffer e.g. take 1 mL of cryptate-antibody stock solution and add it to 49 mL of detection buffer # 3.

4.2. Standard curve preparation

Determine how many samples and replicates will be tested. Each well requires 10 µL of sample or standard.
See concentration on the label.

Standards	Working concentration (M)	Preparation
FLAG-tagged standard stock solution	10×10^{-06}	see label on vial
Std 7	2×10^{-06}	10 µL of stock solution + 40 µL diluent
Std 6	2×10^{-07}	10 µL Std 7 + 90 µL diluent
Std 5	2×10^{-08}	10 µL Std 6 + 90 µL diluent
Std 4	2×10^{-09}	10 µL Std 5 + 90 µL diluent
Std 3	2×10^{-10}	10 µL Std 4 + 90 µL diluent
Std 2	2×10^{-11}	10 µL Std 3 + 90 µL diluent
Std 1	2×10^{-12}	10 µL Std 2 + 90 µL diluent
Std 0	0	90 µL diluent

A recommended standard dilution procedure is listed and illustrated below:

1. Prepare the following serial dilutions:

- Dilute the standard stock solution 5-fold with diluent. E.g. take 10 µL of standard stock solution and add it to 40 µL of diluent, this yields the high standard (Std 7: 2 x 10^{-06} M) for the bottom of the curve.

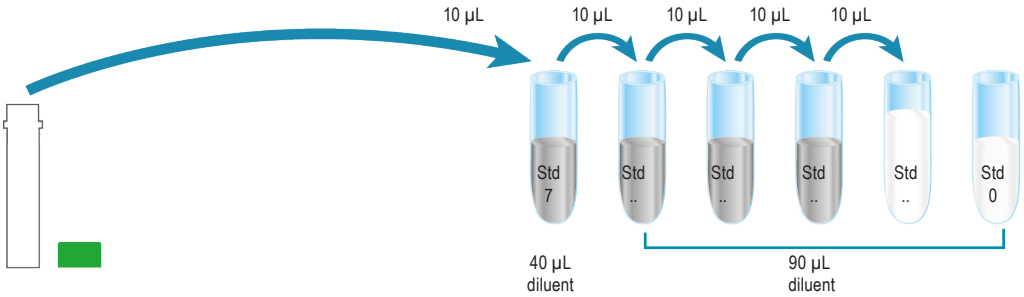
- Use the high standard (Std 7) to prepare the standard curve using 1/10 serial dilutions as follows:

  • Dispense 90µL of diluent in each vial from Std 6 to Std 1.

  • Add 10µL of standard to 90µL of diluent, mix gently and repeat the 1/10 serial dilution to make standard solutions: 2 x 10^{-07}, 2 x 10^{-08}, 2 x 10^{-09}, 2 x 10^{-10}, 2 x 10^{-11}, 2 x 10^{-12} M.

This will create 7 standards for the analyte. Std 0 - Positive control - is diluent alone.

The standard dilution procedure is listed and illustrated below.



5. ASSAY PROTOCOL

Dispense the reagents in the following order:

Carefully follow the order of dispensing the two working detection solutions. Do not pre-mix the d2 and Cryptate solutions prior to dispense.

→ Cover the plate with a plate sealer.

→ Incubate at RT for 2 hours.

→ Remove the plate sealer and,

→ Read the fluorescence emission at two different wavelengths (665nm and 620nm) on a compatible HTRF® reader.

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

Use white plate only, for more information about plates, please visit our website at http://www.cisbio.com/microplate-recommendations

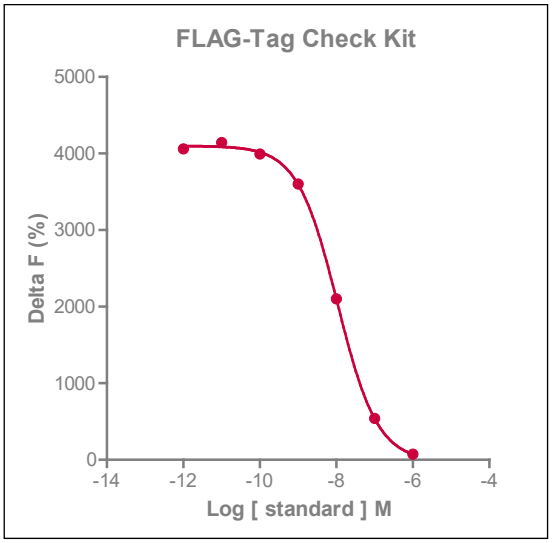
	Assay controls			
	Positive control	Negative control (or Cryptate control)	Buffer control	Sample / Std
	Used to calculate the SignalMax	Used to calculate the delta F % and to check the Cryptate signal at 620nm	used to check background fluorescence	
Sample / Std	-	-	-	10 µL
Diluent	10 µL	10 µL	10 µL	-
FLAG-tagged peptide-d2	5 µL	-	-	5 µL
Anti-FLAG-Eu^{3+}-Cryptate antibody	5 µL	5 µL	-	5 µL
Detection buffer # 3	-	5 µL	10 µL	-

6. DATA REDUCTION

This data must not be substituted for that obtained in the laboratory and should be considered only as an example.
Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration - readouts performed on PHERAstar FS:

[FLAG-tagged peptide]	ratio(1)	CV% (2)	delta F% (3)
Std 0	20141	1%	4312%
Std 1	18987	2%	4059%
Std 2	19375	2%	4144%
Std 3	18686	1%	3993%
Std 4	16902	1%	3602%
Std 5	10049	5%	2102%
Std 6	2924	5%	540%
Std 7	790	4%	73%



To obtain additional information or support, please contact the HTRF technical support team at htrfservices@cisbio.com

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)	$\frac{\text{Ratio standard or sample} - \text{Ratio Negative control}}{\text{Ratio Negative control}} \times 100$	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: http://www.cisbio.com/data-reduction		

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