



MAP-TAU KITS

PROTOCOL

Part # 63ADK008PEG & 63ADK008PEH

Test size#: 500 tests (63ADK008PEG) and 10,000 tests (63ADK008PEH) - assay volume: 20 μ L

Revision: 04-May 2020

Store at: -16°C or below (63ADK008PEG); -16°C or below (63ADK008PEH)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of microtubule-associated protein tau (MAP-Tau) in cell lysates and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, MAP-Tau is detected in a sandwich assay by using anti MAP-Tau antibody labeled with Europium cryptate (donor), and anti MAP-Tau antibody labeled 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). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the MAP-Tau concentration.

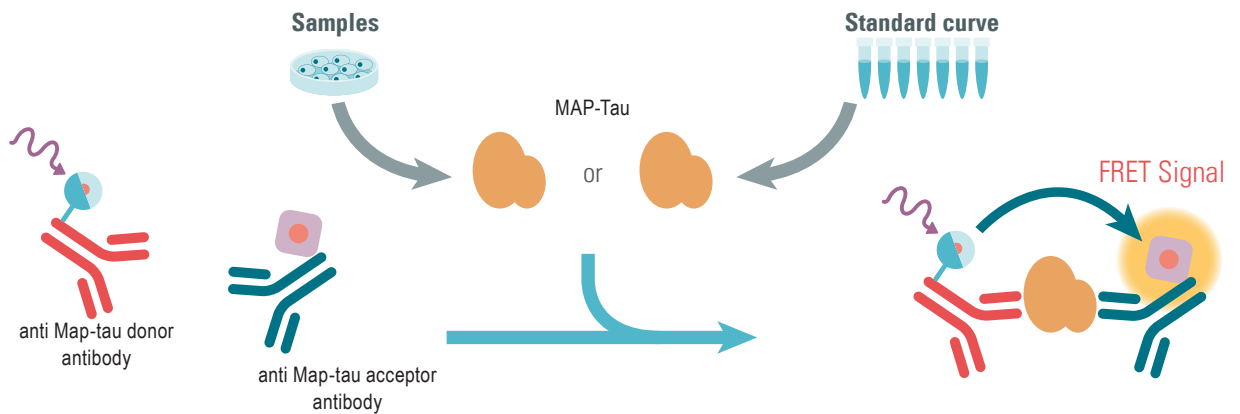
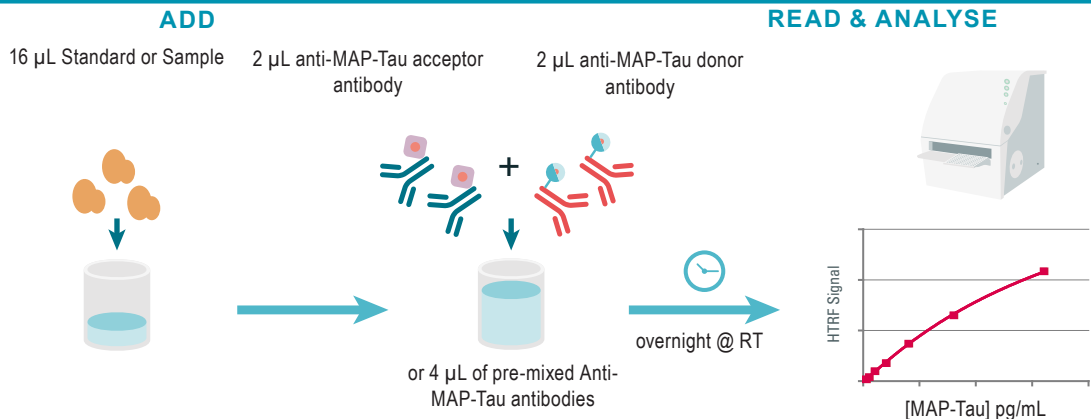


Figure 1: Principle of HTRF MAP-Tau sandwich assay.

PROTOCOL AT A GLANCE



Make sure to use the set-up for Eu Cryptate.

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 63ADK008PEG	10,000 TESTS * CAT # 63ADK008PEH
MAP-Tau Standard Frozen	1 vial - 10 µL 1 mg/mL	1 vial - 10 µL 1 mg/mL
MAP-Tau Eu Cryptate Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
MAP-Tau d2 Antibody	1 vial - 20 µL Frozen - 50X	1 vial - 0.4 mL Frozen - 50X
Lysis buffer ** 4X	1 vial 2 mL	1 vial 130 mL
Detection buffer *** ready-to-use	1 vial 2 mL	1 vial 50 mL

* When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume..

Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

** Medium like cell culture medium can be an alternative to the diluent.

*** The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

PURCHASE SEPARATELY:

- HTRF®-Certified Reader. **Make sure the setup for Eu Cryptate is used.**

For a list of HTRF-compatible readers and set-up recommendations, please visit www.cisbio.com/compatible-readers

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: cisbio.com/microplates-recommendations

STORAGE AND STABILITY

Store the kit at -16°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Detection buffer is shipped frozen, but can be stored at 2-8°C in your premises.



Reagents







If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below .

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect lysis buffer may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Lysis buffer and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- MAP-Tau standards (for standard curve) must be prepared in lysis buffer or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

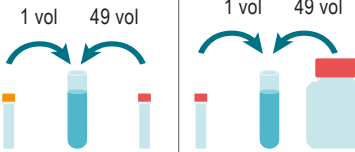


TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 63ADK008PEG			10,000 TESTS KIT - 63ADK008PEH
Anti-MAP-Tau Eu Cryptate antibody			
Thaw the MAP-Tau Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the MAP-Tau Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
Anti-MAP-Tau d2 antibody			
Thaw the MAP-Tau d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.			Thaw the MAP-Tau d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.
MAP-Tau Standard			
Thaw the MAP-Tau Standard in order to obtain a 1 mg/mL stock solution. Mix gently. This stock solution can be frozen and stored at -20°C or below.			Thaw the MAP-Tau Standard in order to obtain a 1 mg/mL stock solution. Mix gently. This stock solution can be frozen and stored at -20°C or below.
Lysis buffer			
Determine the amount of lysis buffer needed for the experiment. Each well requires generally 50 µL of lysis buffer. Dilute 4-fold the 4 X lysis buffer with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 1.25 mL of lysis buffer + 3.75 mL of distilled water). Mix gently after dilution. This 1 X lysis buffer is stable for 2 days at 2-8°C.			Determine the amount of lysis buffer needed for the experiment. Each well requires generally 50 µL of lysis buffer. Dilute 4-fold the 4 X lysis buffer with distilled water: homogenize the 4 X lysis buffer with a vortex and add 1 volume of stock solution in 3 volumes of distilled water (e.g., 1.25 mL of lysis buffer + 3.75 mL of distilled water). Mix gently after dilution. This 1 X lysis buffer is stable for 2 days at 2-8°C.
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 2 µL of MAP-Tau-Eu Cryptate Antibody and 2 µL of MAP-Tau-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 63ADK008PEG			10,000 TESTS KIT - 63ADK008PEH
MAP-Tau Eu Cryptate antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of human-Tau Eu Cryptate antibody with the Detection buffer: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer (e.g., 20 µL of reconstituted Eu Cryptate antibody stock solution + 980 µL of Detection Buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of Anti human MAP-Tau-Cryptate-antibody with the Detection buffer: add 1 volume of Cryptate antibody stock solution in 49 volumes of Detection buffer (e.g., 0.4 mL of reconstituted Cryptate-antibody stock solution + 19.6 mL of Detection Buffer).
MAP-Tau d2 antibody			
Dilute 50-fold the 50X stock solution (thawed reagent) of Human MAP-Tau d2 antibody with the Detection buffer: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer (e.g., 20 µL of reconstituted d2 antibody stock solution + 980 µL of Detection Buffer).			Dilute 50-fold the 50X stock solution (thawed reagent) of Human MAP-Tau d2 antibody with the Detection buffer: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer (e.g., 0.4 mL of reconstituted d2 antibody stock solution + 19.6 mL of Detection Buffer).
Antibody mix			
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).			It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 16 μL of standard.
- Dilute the standard stock solution serially with lysis buffer (1X)
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in lysis buffer (1X).
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

A recommended standard dilution procedure is listed and illustrated below:

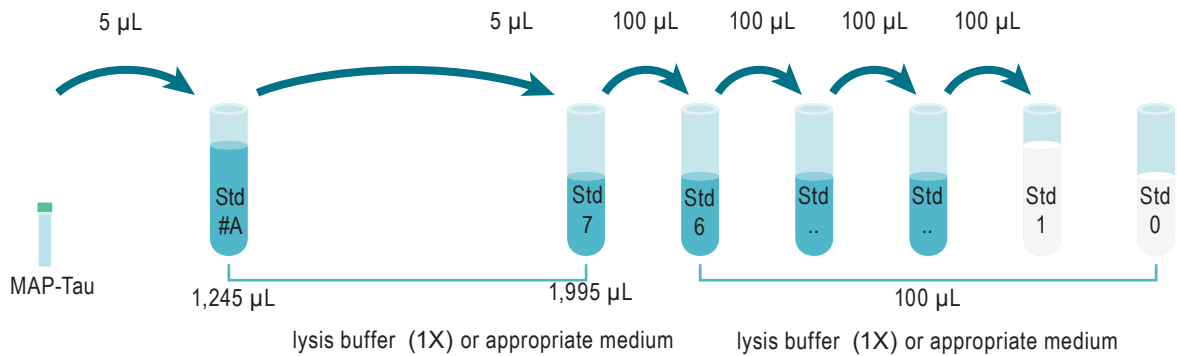
Dilute the standard stock solution 250-fold with lysis buffer; this yields the Intermediate Standard solution #A(4,000,000 pg/mL). E.g: take 5 μL of standard stock solution and add it to 1,245 μL of lysis buffer. Mix gently.

Dilute the Intermediate Standard dilution #A 400-fold with lysis buffer (1X) to prepare high standard (Std 7): e.g. take 5 μL of Intermediate Standard dilution #A and add it to 1,995 μL of lysis buffer (1X). Mix gently.

Use the high standard (Std 7) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 100 μL of lysis buffer (1X) in each vial from Std 6 to Std 0.
- Add 100 μL of standard to 100 μL of lysis buffer (1X), mix gently and repeat the 1/2 serial dilution to make standard solutions: std6, std5, std4, std3, std2, std1.

This will create 7 standards for the analyte. Std 0 (Negative control) is lysis buffer (1X) or appropriate culture medium alone.








STANDARD	SERIAL DILUTIONS	MAP-TAU WORKING SOLUTIONS (pg/mL)
Standard Stock solution	Thawed stock solution	1,000,000,000
Intermediate standard solution #A	5 μL Standard stock solution + 1,245 μL lysis buffer 1X	4,000,000
Standard 7	5 μL Intermediate Standard Solution #A + 1,995 μL lysis buffer 1X	10,000
Standard 6	100 μL standard 7 + 100 μL lysis buffer 1X	5,000
Standard 5	100 μL standard 6 + 100 μL lysis buffer 1X	2,500
Standard 4	100 μL standard 5 + 100 μL lysis buffer 1X	1,250
Standard 3	100 μL standard 4 + 100 μL lysis buffer 1X	625
Standard 2	100 μL standard 3 + 100 μL lysis buffer 1X	312.5
Standard 1	100 μL standard 2 + 100 μL lysis buffer 1X	156
Standard 0	100 μL lysis buffer 1X	0

TO PREPARE SAMPLES:

- Each well requires 16 μ L of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 7) must be diluted lysis buffer (1X)
- The assay can be run under a two-plate protocol, where cells are plated and stimulated in the same culture plate, then transferred to the assay plate for the HTRF® detection. This protocol enables the cells' viability and confluence to be monitored. It can also be further streamlined to a one-plate assay protocol where plating, stimulation and detection is performed in a single plate. For two-plate & one-plate assay protocols for suspension cells and adherent cells kept in medium for the lysis, we recommend to use the lysis buffer 4X (ready to use) For two-plate & one-plate assay protocols for adherent cells removing the medium for lysis, we recommend to use the lysis buffer 1X. Cell density, stimulation time, lysis step and other parameters related to the biology are cell-dependent and need to be optimized.
- To obtain additional information or support, please contact the HTRF technical support team at cisbio.com/contact-us

ASSAY PROTOCOL

		Standard (Std 0 - Std 7)	Samples
Step 1		Dispense 16 μ L of each MAP-Tau standard (Std 0 - Std 7) into each standard well	Dispense 16 μ L of each sample into each sample well
Step 2		Add 2 μ L of MAP-Tau d2 antibody working solution to all wells	
Step 3		Add 2 μ L of MAP-Tau Eu Cryptate antibody working solution to all wells	
Step 4		Seal the plate and incubate overnight @ RT	
Step 5		Remove the plate sealer and read on an HTRF® compatible reader	

DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

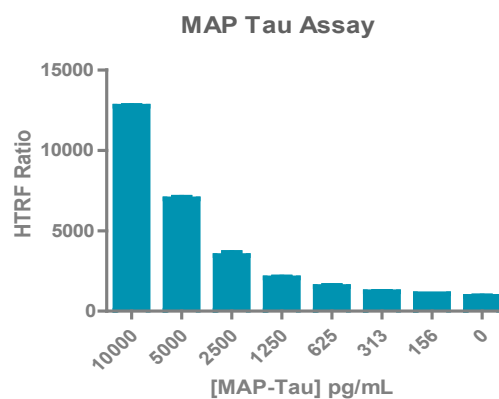
For more information about data reduction, please visit <http://www.cisbio.com/htrf-ratio-and-data-reduction>

RESULTS

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

	Ratio ⁽¹⁾	CV ⁽²⁾
Standard 7 -10,000 pg/mL	12,747	0.5%
Standard 6 - 5,000 pg/mL	6,979	2.5%
Standard 5 - 2,500 pg/mL	3,462	9.0%
Standard 4 - 1,250 ng/mL	2,069	3.7%
Standard 3 - 625 pg/mL	1,530	7.0%
Standard 2 - 312.5 pg/mL	1,212	2.1%
Standard 1 - 156 pg/mL	1,082	0.7%
Standard 0 - Negative control	920	4.8%



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Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880

Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors



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