MANUAL

HUMAN AMYLOÏD BETA PEPTIDE (1-40) KITS

Part # 62B40PEG & 62B40PEH

Test size#: 500 tests (62B40PEG) and 10,000 tests (62B40PEH) - assay volume: 20 µL

Revision: #05 of September 2023

Store at: 2-8°C (62B40PEG); - 60°C or below (62B40PEH)

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

ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of Amyloïd beta peptide (1-40) in cell supernatants and whole cells 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, Amyloid β peptide (1-40) is detected in a sandwich assay by using anti Amyloid β peptide (1-40) antibody labeled with Europium cryptate (donor), and anti Amyloid β peptide (1-40) antibody labeled with XL665 (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 Amyloid β peptide (1-40) concentration.

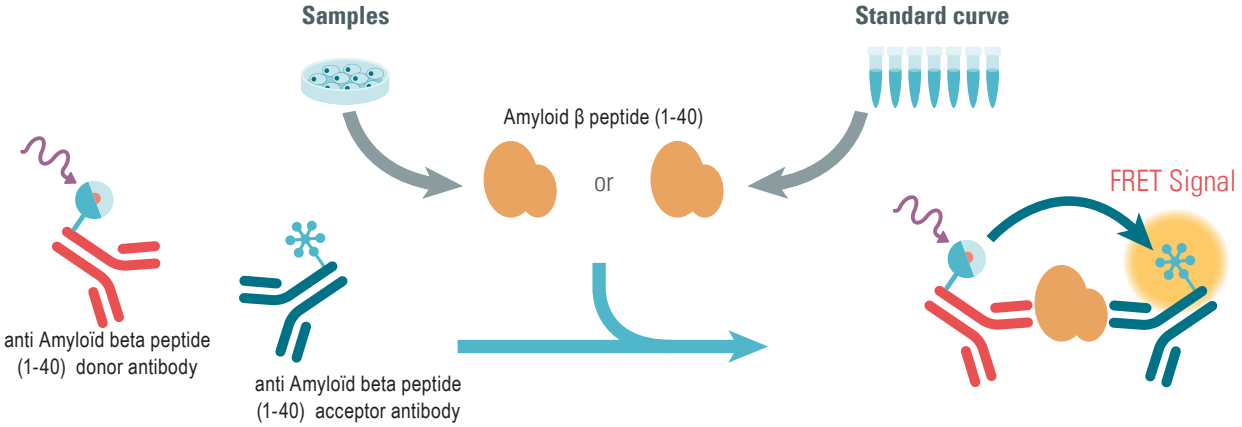
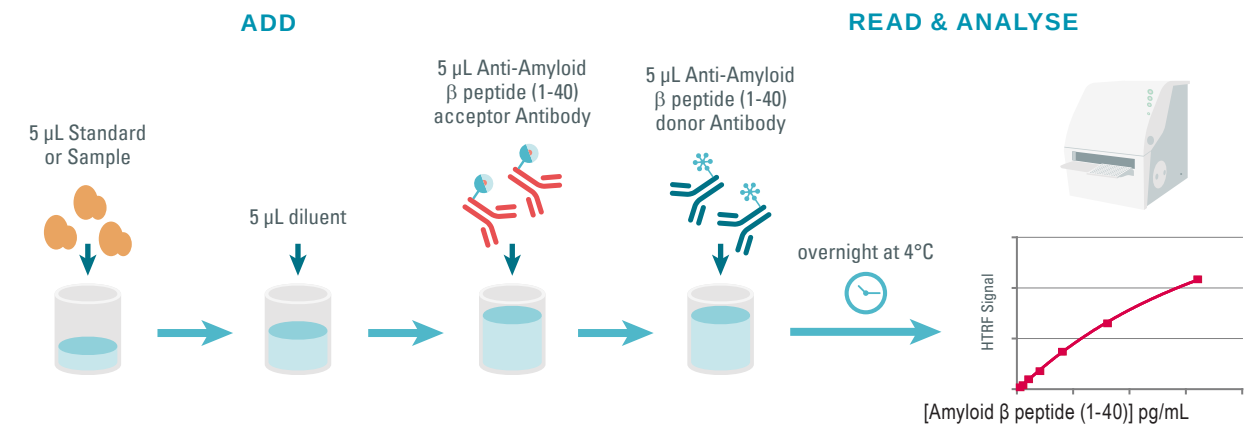


Figure 1: Principle of HTRF Amyloid β peptide (1-40) sandwich assay.

MANUAL AT A GLANCE



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

MATERIALS PROVIDED:

KIT COMPONENTS	500 TESTS * CAT # 62B40PEG	10,000 TESTS * CAT # 62B40PEH
Amyloid β peptide (1-40) Standard Lyophilized	1 vial	1 vial
Amyloid β peptide (1-40) Eu Cryptate Antibody	1 vial Lyophilized	1 vial - 1 mL Frozen - 50X
Amyloid β peptide (1-40) XL665 Antibody	1 vial Lyophilized	1 vial - 1 mL Frozen - 50X
Diluent ** ready-to-use	1 vial 20 mL	1 vial 20 mL
Detection buffer *** ready-to-use	1 vial 7 mL Detection Buffer #2	1 vial 105 mL Detection Buffer #3

* 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.revvity.com

- Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: www.revvity.com

STORAGE AND STABILITY

Store the kit 62B40PEG at 2-8°C and the kit 62B40PEH at - 60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label.

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 -60°C or below .

Volume of Human Amyloïd beta peptide (1-40) standard aliquots should not be under 20 µL.

REAGENT PREPARATION

BEFORE YOU BEGIN:

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Allow the lyophilized reagents to warm up to room temperature for at least 30 mins before reconstitution and thaw the frozen reagents at room temperature, allow them to warm up.
- Before use, allow Diluent 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.
- Amyloid β peptide (1-40) standards (for standard curve) must be prepared in diluent 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.

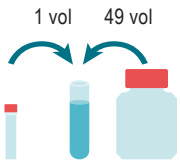
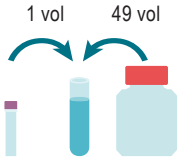
TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 62B40PEG			10,000 TESTS KIT - 62B40PEH
Anti-Amyloid β peptide (1-40) Eu Cryptate antibody			
Reconstitute the Amyloid β peptide (1-40) Eu Cryptate antibody with 2.5 mL detection buffer #2. Mix gently. This ready-to-use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen for 4 days at 4°C.			Thaw the Amyloid β peptide (1-40) Eu Cryptate antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen for 4 days at 4°C.
Anti-Amyloid β peptide (1-40) XL665 antibody			
Reconstitute the Amyloid β peptide (1-40) XL665 antibody with 2.5 mL detection buffer #2. Mix gently. This ready-to-use 1X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen for 4 days at 4°C.			Thaw the Amyloid β peptide (1-40) XL665 antibody . Mix gently. This 50X stock solution can be frozen and stored at -60°C or below. It can be stored unfrozen for 4 days at 4°C.
Amyloid β peptide (1-40) Standard			
Reconstitute the Peptide β (1-40) standard with distilled water in order to obtain a 8000 pg/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This unused calibrator stock solution is stable 4 days at 4°C. It can be frozen (at -60°C) and thawed once only.			Reconstitute the Peptide β (1-40) standard with distilled water in order to obtain a 8000 pg/mL stock solution. See instructions on vial label for reconstitution volume. Mix gently after reconstitution. This unused calibrator stock solution is stable 4 days at 4°C. It can be frozen (at -60°C) and thawed once only.
Diluent			
The diluent is ready-to-use.			The diluent is ready-to-use.
Detection buffer			
The Detection buffer is ready-to-use.			The Detection buffer is ready-to-use.

TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5 µL of Amyloid β peptide (1-40)-Eu Cryptate Antibody and 5 µL of Amyloid β peptide (1-40)-XL665 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 62B40PEG			10,000 TESTS KIT - 62B40PEH
Amyloid β peptide (1-40) Eu Cryptate antibody			
After reconstitution, the peptide β (1-40) Eu Cryptate antibody is ready-to-use.			Dilute 50-fold the 50X stock solution (thawed reagent) of peptide β (1-40) Eu Cryptate body stock solution with the Detection buffer #3: add 1 volume of Eu Cryptate body stock solution in 49 volumes of Detection buffer #3 (e.g., 1 mL of Eu Cryptate body stock solution + 49 mL of Detection Buffer #3).
Amyloid β peptide (1-40) XL665 antibody			
After reconstitution, the peptide β (1-40) XL665 antibody is ready-to-use.			Dilute 50-fold the 50X stock solution (thawed reagent) of peptide β (1-40) XL 665 antibody with the Detection buffer #3: add 1 volume of XL665 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 1 mL of XL665 antibody stock solution + 49 mL of Detection buffer #3).
Antibody mix			
Do not pre-mix the two ready-to-use antibody solutions.			Do not pre-mix the two ready-to-use antibody solutions.

TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 5 µL of standard.
- Dilute the standard stock solution serially with diluent
- 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 diluent .
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

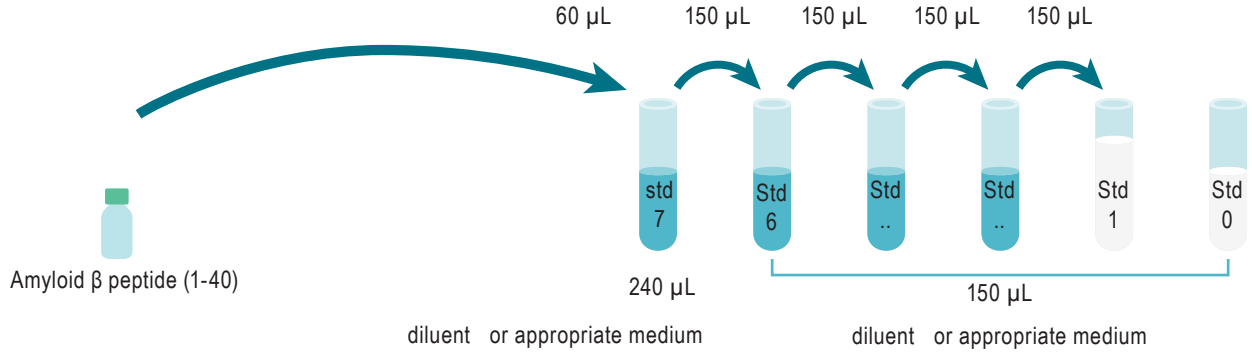
A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 5-fold with diluent to prepare high standard (std 7): e.g. take 60 µL of standard stock solution and add it to 240 µL of diluent . Mix gently.

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

- Dispense 150 µL of diluent in each vial from Std 6 to Std 0.
- Add 150 µL of standard to 150 µL of diluent , 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 diluent or appropriate culture medium alone.



STANDARD	SERIAL DILUTIONS	HUMAN AMYLOÏD BETA PEPTIDE (1-40) WORKING SOLUTIONS (pg/mL)
Standard Stock solution	Reconstituted lyophilisate	8,000
Standard 7	60 µL Standard stock solution + 240 µL Diluent	1,600
Standard 6	150 µL standard 7 + 150 µL Diluent	800
Standard 5	150 µL standard 6 + 150 µL Diluent	400
Standard 4	150 µL standard 5 + 150 µL Diluent	200
Standard 3	150 µL standard 4 + 150 µL Diluent	100
Standard 2	150 µL standard 3 + 150 µL Diluent	50
Standard 1	150 µL standard 2 + 150 µL Diluent	25
Standard 0	150 µL Diluent	0

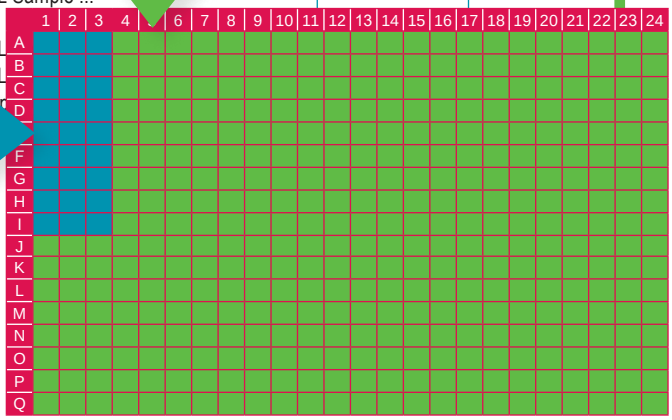
TO PREPARE SAMPLES:

- Each well requires 5 µL 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 diluent

ASSAY MANUAL

	Standard (Std 0 - std 7)	Samples
Step 1 	Dispense 5 µL of each Amyloid β peptide (1-40) standard (Std 0 - std 7) into each standard well And add 5 µL diluent	Dispense 5 µL of each sample into each sample well And add 5 µL diluent
Step 2 	Add 5 µL of Amyloid β peptide (1-40) XL665 antibody working solution to all wells	
Step 3 	Add 5 µL of Amyloid β peptide (1-40) Eu Cryptate antibody working solution to all wells	
Step 4 	Seal the plate and incubate overnight @ 4°C	
Step 5	Remove the plate sealer and read on an HTRF® compatible reader	

	1	2	3	4	5	6
A	5 µL Std 0 (Negative control) 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well A1	Repeat Well A1	5 µL Sample 1 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well A4	Repeat Well A4
B	5 µL Std 1 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well B1	Repeat Well B1	5 µL Sample 2 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well B4	Repeat Well B4
C	5 µL Std 2 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well C1	Repeat Well C1	5 µL Sample 3 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well C4	Repeat Well C4
D	5 µL Std ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well D1	Repeat Well D1	5 µL Sample ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well D4	Repeat Well D4
E	5 µLStd ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well E1	Repeat Well E1	5 µL Sample ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well E4	Repeat Well E4
F	5 µL Std ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well F1	Repeat Well F1	5 µL Sample ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well F4	Repeat Well F4
G	5 µL Std ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well G1	Repeat Well G1	5 µL Sample ... 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well G4	Repeat Well G4
H	5 µL Std ... 5 µL diluent 5 µL Amyloid β peptide (1-40)-XL665 5 µL Amyloid β peptide (1-40)-Eu Cryptate	Repeat Well H1	Repeat Well H1	5 µL Sample ... 5 µL 5 µL		



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.

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

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

$$\text{delta F (\%)} = \frac{\text{Ratio Standard or sample - Ratio Negative Control}}{\text{Ratio Negative Control}} \times 100$$

For more information about data reduction, please visit www.revvity.com

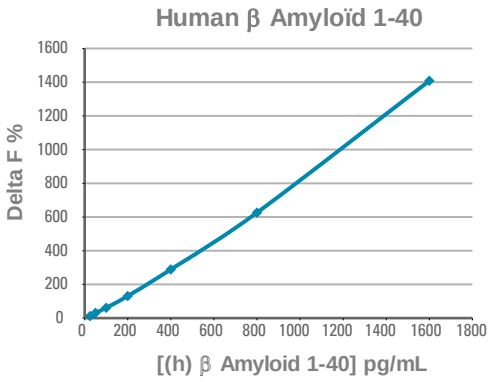
RESULTS

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.

Delta F obtained for samples can be reported on the calibration curve to deduce respective amyloïd peptide β (1-40) concentration:

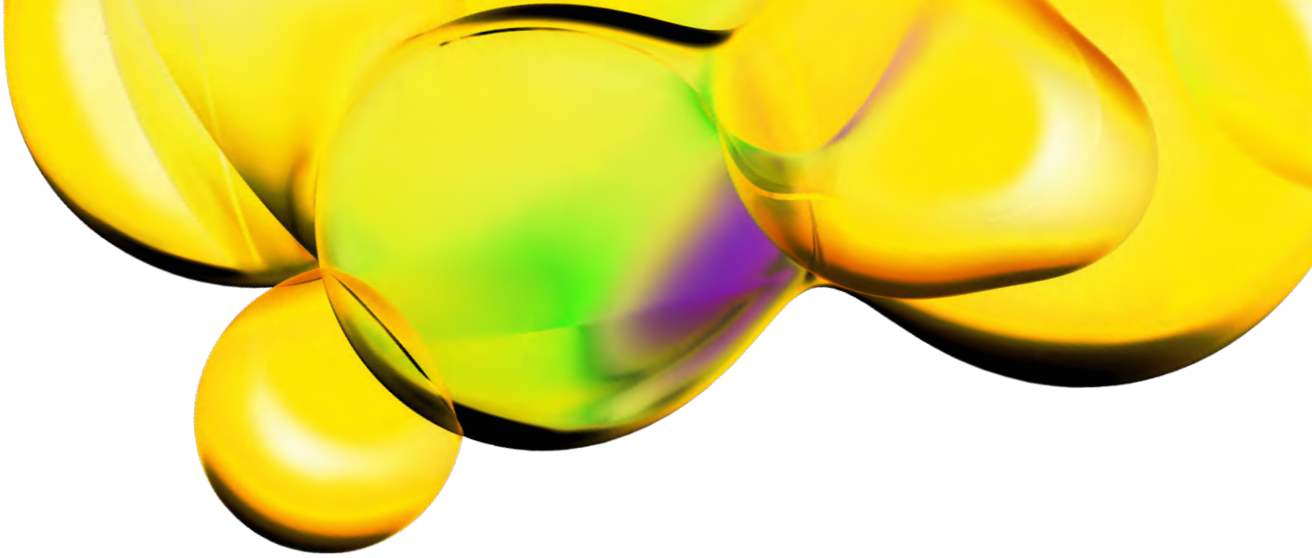
	Ratio (1)	CV (2)	Delta F% (3)
Standard 0 - Negative control	390	4.6%	
Standard 1 - 25 pg/mL	437	4.2%	12%
Standard 2 - 50 pg/mL	512	2.2%	31%
Standard 3 - 100 pg/mL	627	3.4%	61%
Standard 4 - 200 pg/mL	899	0.3%	131%
Standard 5 - 400 pg/mL	1,517	2.6%	289%
Standard 6 - 800 pg/mL	2,827	1.1%	626%
Standard 7 - 1,600 pg/mL	5,872	1.5%	1,408%



ANALYTICAL CHARACTERISTICS

DETECTION LIMIT

	Diluent	Culture medium
Detection limit(Std 0 + 2SD)	≤ 10 pg/mL	≤ 30 pg/mL



Manufactured by Cisbio Bioassays - Parc Marcel Boiteux - 30200 Codolet – FRANCE

www.revvity.com

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com