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HTRF® package insert

Document reference : 62B40PEB rev05 (July 2008)

Amyloïd β 1-40 peptide

For in vitro research use only
Storage temperature : 2-8°C

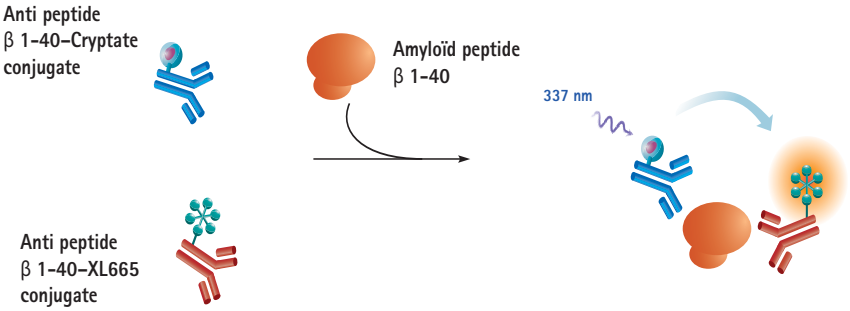
Packaging details :

	384-well low volume plate (20 µl)
62B40PEB	1,000 tests

1. Assay description and intended use

This kit is intended for the quantitative determination of amyloid β peptide (1-40). It is a single step homogeneous immunoassay. (For in-vitro research use only)

Its principle is based on HTRF® technology. As shown below, peptide β (1-40) is detected by a MAb anti-peptide β (1-40) labeled with Cryptate , the second MAb is labeled with XL665. Specific signal (i.e. energy transfer) is proportional to the concentration of amyloïd peptide β (1-40) in the sample or standard.



2. Protocol

2.1. Supplied reagents and preparation

Supplied reagents	Reagent preparation
Anti-peptide β (1-40)-Cryptate conjugate** 1 vial lyophilized*	Add 5mL of reconstitution buffer to each vial. Mix gently.
Anti-peptide β (1-40)-XL665 conjugate 1 vial lyophilized*	
Peptide β (1-40) calibrator 1 vial, lyophilized*	Reconstitute with distilled water. See label indications for reconstitition volume. Mix gently. See calibration curve preparation (§2.3.)
Reconstitution buffer (1 vial of 13 mL) *50 mM Phosphate buffer, pH 7.0, 0.8M KF*	
Diluent (1 vial of 20 mL) *50 mM Phosphate buffer, pH 7.0, 0.2 % BSA, NaN_3, preservatives*	

Allow the reagent to warm up at room temperature at least 30 mins.

*All reagents were lyophilized in 50 mM Phosphate buffer, pH7, containing BSA protease free and stabilizers.

**The Cryptate conjugate concentration was optimized in order to ensure an average counting of 40,000 cps at 620 nm (384-well low volume format), using the reference RUBYstar reader (BMG LABTECH).

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.

2.2. Reagent storage and stability

All reagents should be stored at 2-8°C until reconstituted. Once reconstituted, unused reagents are stable 4 days at 4°C. They can be refrozen (-80°C) and thawed once only.

2.3. Calibration curve preparation

Reconstitute the maximum calibrator according to the indications printed on the label and follow the dilution sequence shown in the following table to draw up the calibration

Calibrator	Calibrator concentration in pg/mL	Preparation
Stock solution	8000	Reconstituted calibrator
Cal 7 (max calibrator)	1600	60 µL stock solution + 240 µL diluent
Cal 6	800	150 µL Cal 7 + 150 µL diluent
Cal 5	400	150 µL Cal 6 + 150 µL diluent
Cal 4	200	150 µL Cal 5 + 150 µL diluent
Cal 3	100	150 µL Cal 4 + 150 µL diluent
Cal 2	50	150 µL Cal 3 + 150 µL diluent
Cal 1	25	150 µL Cal 2 + 150 µL diluent
Cal 0	0	150 µL diluent

2.4. Sample preparation

Dilute all samples to be assayed with the diluent (PO4 50 mM, BSA 0.2 % pH 7.0).Consecutive dilutions should be made within the 0-1600 pg/mL range (working solution).

2.5. Distribution in 384-well low volume plate (20 µL)

Dispense the reagents in the following order :

- 5 µL sample or calibrator *
- 5 µL diluent
- 5 µL anti peptide β (1-40) - Cryptate conjugate
- 5 µL anti peptide β (1-40) - XL665 conjugate

*for the negative control, replace the calibrator by 5 µL of diluent.

Cover the plate with a plate sealer and let the incubation take place at 4°C (24 hours).

Remove the plate sealer and read on a compatible HTRF® reader (more information about compatible reader at htrf.com / readers).

3. Data reduction and example of a calibration curve

This data should not be substituted for results obtained in the laboratory. Results are calculated from 665 nm/620 nm ratio and expressed in delta F.

	A (665nm)	B (620nm)	Ratio (1)	Mean Ratio (2)	CV % (3)	Delta F % (4)
Negative control	1780 1750	47092 47106	378 372	374	1.2	
[peptide β 1-40] pg/mL initial						
25	2182 2229	47274 48521	462 459	460	0.3	23
50	2509 2419	46644 45719	538 529	533	1.2	42
100	2987 2924	46897 46014	637 635	636	0.2	70
200	4042 4317	45657 46918	885 920	903	2.7	141
400	6459 6733	46527 45961	1388 1465	1427	3.8	281
800	12899 12679	46687 46457	2763 2729	2746	0.9	633
1600	24467 24172	44613 45054	5484 5365	5424	1.6	1348

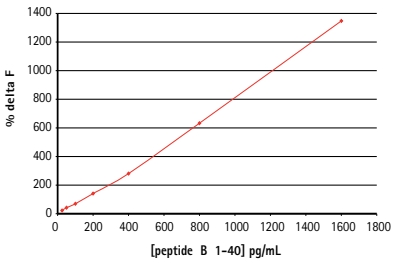
1. $\text{Ratio} = \frac{A_{665nm}}{B_{620nm}} \times 10^4$

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

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

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

(Ratio_{neg} = negative control)



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

4. Detection limit

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

5. Assay flexibility and miniaturization

When used as suggested, the kit will provide sufficient reagents for 1,000 tests 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 10µL), 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, 2 times less 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 format		
Assay components	**Volume proportion**	**1536-well (10 µL)**	**384-well low volume (20 µL)**	**96 half-well (100 µL)**
Sample	1 volume	2.5 µL	5 µL	25 µL
Diluent	1 volume	2.5 µL	5 µL	25 µL
Cryptate conjugate	1 volume	2.5 µL	5 µL	25 µL
XL665 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).