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Amyloid β 1-40 peptide 20,000 tests

For in vitro research use only
 Reagent storage temperature: -80°C

Packaging details :

	384-well low volume plate (20 μl)
62B40PEC	20,000 tests

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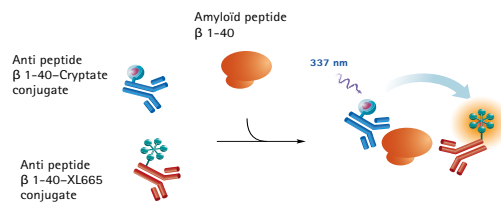
HTRF[®] package insert

Document reference : 62B40PEC rev13 (August 2011)

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 amyloid peptide β (1-40) in the sample or standard.



2. Protocol

2.1. Supplied reagents and preparation

Allow the reagents to come to room temperature before use.

Supplied reagents	Reagent preparation (working solutions)
Anti-peptide β (1-40)-Cryptate conjugate 1 vial of 1mL (frozen)	Take 1mL of stock solution and add it to 99 mL of reconstitution buffer. Mix gently.
Anti-peptide β (1-40)-XL665 conjugate 1 vial of 1 mL (frozen)	
Peptide β (1-40) calibrator 1 vial (lyophilized)	Reconstitute with distilled water. See label indications for reconstitution volume. Mix gently. See calibration curve preparation (S2.3.)
Reconstitution buffer (1 vial of 200 mL) 50 mM Phosphate buffer, pH 7.0, 0.8M KF, 0.2% BSA (frozen)	
Diluent (1 vial of 20 mL) 50 mM Phosphate buffer, pH 7.0, 0.2 % BSA, 0.02% NaN ₃ , preservatives (frozen)	

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

Lyophilized calibrator, diluent and reconstitution buffer can be stored at $2-8^{\circ}\text{C}$ before use.

The two conjugates must be frozen at -80°C before use.

Once reconstituted, unused calibrator is stable 4 days at 4°C . It can be refrozen (at -20°C) and thawed once.

Once thawed, unused conjugates 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 curve. Dilution must be carried out with the diluent (or with freshly made P04 50 mM, BSA 0.2% pH7).

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	1667 1644	41450 43626	402 377	390	4.6	
[peptide β 1-40] pg/mL initial						
25	1813 1727	40262 40687	450 424	437	4.2	12
50	2065 2122	40956 40819	504 520	512	2.2	31
100	2774 2371	43220 38772	642 612	627	3.4	61
200	3905 3441	43333 38340	901 897	899	0.3	131
400	6574 6513	44174 42155	1488 1545	1517	2.6	289
800	11452 11730	40823 41188	2805 2848	2827	1.1	626
1600	22262 22301	38307 37592	5811 5932	5872	1.5	1408

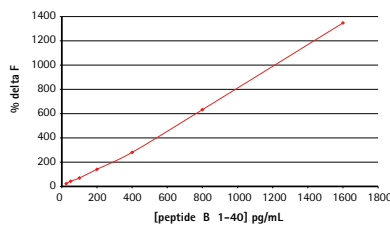
$$1. \text{Ratio} = \frac{A_{665\text{nm}}}{B_{620\text{nm}}} \times 10^4$$

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

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

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

(Ratio_{neg} = negative control)



Delta F obtained for samples can be reported on the calibration curve to deduce respective amyloid 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 components	Volume proportion	Assay format		
		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
		40.000 tests	20.000 tests	4.000 tests

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