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Aβ ELISA

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We use this protocol and it's working

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Abstract

Measure Aβ42 levels in human neurons.

1 Human Aβ42 ELISA Kit (Thermo Fisher Scientific).

2 Human tNeurons are first trypsinized, washed with ice-cold PBS and pelleted by centrifugation for 5 min at 1,000 X G.

3 Cells are lysed with RIPA buffer (25 mM Tris-HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% sodium deoxycholate) supplemented with protease inhibitors (Roche).

4 Collect the supernatants and protein concentrations are determined by BCA assay (Thermo Fisher Scientific).

5 Human Aβ42 ELISA Kit is used to detect and quantify the Aβ42 levels in total tNeuron lysates.

6 Prepare standards of 1000, 500, 250, 125, 62.5, 31.25, 15.63, and 0 pg/mL human Aβ42 in Standard Diluent Buffer.

7 50 µL of standards, control medium, cell lysates are added to each well of a 96-well plate.

8 Add 50 µL anti-human Aβ42 antibody per well and incubate 3 hr in the dark at room temperature with shaking.

9 Remove the solution and rinse wells 4 times with 1X Wash Buffer.

10 Add 100 µL Anti-Rabbit IgG HRP into each well and incubate in the dark for 30 min at room temperature.

11 Remove the solution and rinse wells 4 times with 1X Wash Buffer.

12 Add 100 µL Stabilized Chromogen to each well and incubate in the dark for 30 min at room temperature.

13 Add 100 µL Stop Solution to each well. The plate is analyzed within 2 hr according to manufacturer's protocol and Aβ42 values are normalized to total protein concentration of lysates.