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Sandwich ELISA for the quantification of alpha-synuclein

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Protocol status: Working
We use this protocol and it's working.

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Abstract

This protocol details the procedure to quantify total alpha-synuclein from tissue and biological fluids by ELISA.

Attachments

gegmbqbhp.pdf
123KB

Materials

Materials:

Coating buffer:

	A	B
	NaHCO3	200 mM
	Sodium azide (pH9.6)	0.02%

Blocking buffer:

	A	B
	PBS	
	Gelatin	2.50 %
	Tween	0.03 %

Sandwich ELISA for alpha-synuclein

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Note

Note: Antibody pairing determined based on sample type and should be optimized.

Coating - Day 1

2 Coat 384-well plate with primary antibody.

2.1 Make Coating buffer [M] 200 millimolar (mM) $NaHCO_3$ with 0.02% sodium azide (pH 9.6).

2.2 1^0 antibody most used is syn1 at 1:500 dilution (BD 610787).

3 Add 25 µL per well (avoiding outer wells).

4 Incubate on rocker at 4 °C Overnight .

Blocking - Day 2

5 Make blocking buffer (PBS+2.5% gelatin, 0.025% tween).

6 Wash plate 3X using 1xPBS-T in plate washer.

7 Blot on paper towel.

8 Add 80 µL per well of blocking buffer.

9 Incubate at 37 °C for 01:00:00 . 1h

10 During incubation, prepare standards and samples On ice , dilute in blocking buffer (BB).

Samples- Day 2

11 Standards – use full length recombinant alpha-synuclein. Stock aliquoted upon opening.

11.1 Make serial dilutions in blocking buffer, starting from [M] 10 Mass Percent stock.

12 Dilute samples in blocking buffer.

13 Wash plate 3X, blot on paper towel.

14 Add 25 µL per well of standards and samples (in duplicate).

15 Incubate at Room temperature for 02:00:00 . 2h

Detection antibody - Day 2

16 Bring blocking buffer to Room temperature before use.

17 Dilute detection antibody in blocking buffer.

- 2 ^{o} antibody most used is biotinylated-hSA4, at 1:200 dilution (commercially MJFR1 ab138501; biotinylated in-lab).

18 Wash plate 3X, blot on paper towel.

19 Add 25 µL per well of detection antibody.

20 Incubate at 37 °C for 01:00:00 . 1h

Avidin-alkaline phosphatase - Day 2

21 Wash plate 5X, blot on paper towel.

22 Use avidin-alkaline phosphatase at [M] 0.6 Mass Percent of blocking buffer.

23 Add 25 µL per well of AAP.

24 Incubate at 37 °C for 00:30:00 . 30m

Substrate - Day 2

1h 2m 30s

25 Make pNPP - 1 of each tablet for 5 mL ddH_2O (cover tube in aluminum, rock to dissolve).

26 Wash plate 5X, blot on paper towel.

27 Add 25 µL /well of pNPP, read plate immediately.

27.1 Read absorbance at 405nm, every 2.5 minutes for 01:00:00 . 1h

27.2 Use data from a read when the standards are linear.