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\bigcirc ELISA (enzyme-linked immunosorbent assay) for α -Syn quantification

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

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ABSTRACT

ELISA (enzyme-linked immunosorbent assay) for α -Syn quantification. Useful for analyses of alpha-Synuclein and related proteins in the context of synucleinopathy.

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1	Coat 384-well MaxiSorp plates (Nunc, Inc) with capturing antibody (α-Syn, BD Biosciences, 610787) diluted 1:500 in coating buffer (NaHCO3 with 0.2% NaN3, pH9.6) overnight at 4 °C.
2	Wash 3 times with PBS.0.05% Tween-20 (PBS-T).
3	Block for 1hr at 37 °C in blocking buffer (1.125% fish skin gelatin; PBS-T)
4	Load samples in duplicate into wells.
5	Incubate at RT for 2hr.
6	Add the desired antibody to the plate for 1 h at 37 °C

7 Wash 5 times with PBS-T.

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8	Add ExtrAvidin phosphatase (Sigma, E2636) diluted in blocking buffer to wells. Incubate for 30 min at 37 °C.
9	Carry out colour development with fast-p-nitrophenyl phosphate (Sigma, N1891) at 405 nm every 2.5 min for up to 60 min.
10	Identify time point(s) where standards and sample dilutions are in the log phase.

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