MSD® MULTI-ARRAY Assay System

Human α-Synuclein Kit

1-Plate Kit	K151TGD-1
5-Plate Kit	K151TGD-2
25-Plate Kit	K151TGD-4

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MSD Neurodegenerative Disease Assays

Human α-Synuclein Kits

For use with human cerebrospinal fluid (CSF) and serum.

This package insert must be read in its entirety before using this product.

FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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Ordering Information

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Introduction

Alpha-synuclein is a 140 amino acid protein abundantly expressed in the nervous system and genetically linked to Parkinson's disease (PD).[1,2] It is thought to maintain synaptic integrity and normal cellular homeostasis through synaptic vesicle recycling and neurotransmitter release modulation.[1] While native α-synuclein is unfolded, it has a propensity to form toxic soluble oligomers (i.e., protofibrils) that ultimately aggregate into insoluble fibrils termed Lewy bodies (LBs). Aberrant α-synuclein pathology is prevalent among neurological samples from patients with α-synuclein-related conditions, commonly referred to as "synucleinopathies." These disorders include PD, dementia with LBs (DLB), and multiple-system atrophy (MSA).[3] Alpha-synuclein has been detected in several biological matrices, such as CSF, serum, plasma, and whole blood.[2,4] Biomarkers that can effectively detect early or pre-symptomatic disease and distinguish PD incidence from other neurodegenerative conditions are of high interest. The MSD Human α-Synuclein Kit can be used to measure α-synuclein in human CSF and serum.

Principle of the Assay

MSD neurodegenerative disease assays provide a rapid and convenient method for measuring the levels of protein targets within a single, small-volume sample. Human α-Synuclein is a sandwich immunoassay. MSD provides a plate pre-coated with capture antibodies on independent and well-defined spots in the layout shown below. The user adds the sample and a solution containing detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG™) over the course of one or more incubation periods. Analytes in the sample bind to capture antibodies immobilized on the working electrode surface; recruitment of the detection antibodies by the bound analytes completes the sandwich. The user adds an MSD buffer that provides the appropriate chemical environment for electrochemiluminescence and loads the plate into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light to provide a quantitative measure of analyte in the sample.

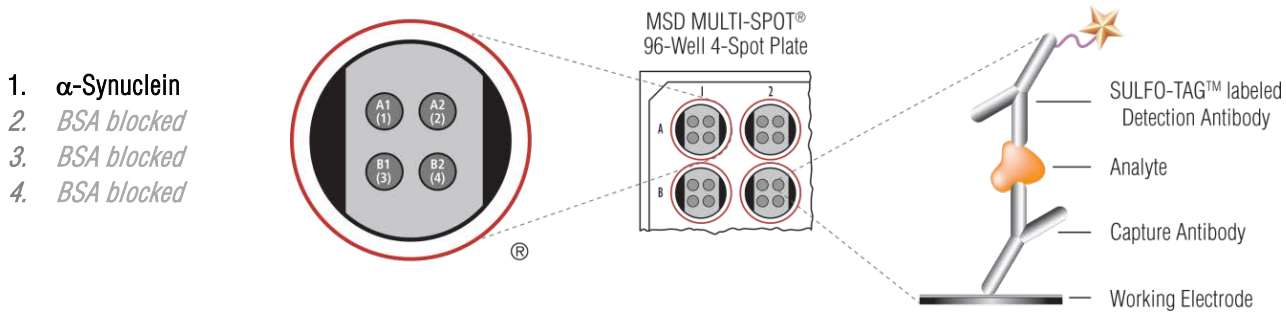


Figure 1. Spot diagram showing placement of analyte capture antibodies for the Human α-Synuclein Kit. The numbering convention for the different spots is maintained in the software visualization tools, on the plate packaging, and in the data files.

Reagents Supplied

Reagent	Storage	Catalog #	Size	Quantity Supplied			Description
				1 Plate Kit	5 Plate Kit	25 Plate Kit	
MULTI-SPOT® 96-well 4-spot Human α-Synuclein Plate	2–8°C	N451TGA-1	4-spot	1	5	25	96-well plate, foil sealed with desiccant.
SULFO-TAG Anti-α-Synuclein Antibody (50X) [1]	2–8°C	D21TG-2	75 µL	1 vial			SULFO-TAG–conjugated antibody
		D21TG-3	375 µL		1 vial	5 vials	
α-Synuclein Calibrator (20X)	≤-70°C	C01TG-2	30 µL	1 vial	5 vials	25 vials	Recombinant α-synuclein protein in a buffered protein diluent.
Diluent 35	2–8°C	R50AE-3	30 mL	1 bottle			Diluent for samples and calibrator; contains blockers and preservatives.
		R50AE-2	150 mL		1 bottle	5 bottles	
Diluent 100	2–8°C	R50AA-4	50 mL	1 bottle	1 bottle	5 bottles	Diluent for detection antibody; contains protein, blockers, and preservatives.
Blocker D–G [2]	≤-10°C	R93BH-3	1.0 mL	1 vial	2 vials	10 vials	Goat gamma globulin solution
Blocker D–M [2]	≤-10°C	R93BM-1	200 µL	1 vial			Mouse gamma-globulin solution
		R93BM-2	900 µL		1 vial	5 vials	
Blocker D–R [2]	≤-10°C	R93BR-1	50 µL	1 vial			Rabbit gamma-globulin solution
		R93BR-2	200 µL		1 vial	5 vials	
Read Buffer T (4X)	RT	R92TC-3	50 mL	1 bottle	1 bottle	5 bottles	MSD buffer to catalyze the electro-chemiluminescence reaction

Additional Materials and Equipment

- ❑ Appropriately sized tubes for reagent preparation
- ❑ Polypropylene microcentrifuge tubes for preparing dilutions
- ❑ Liquid handling equipment for desired throughput, capable of dispensing 10 to 150 µL/well into a 96-well microtiter plate
- ❑ Plate washing equipment: automated plate washer or multichannel pipette
- ❑ Microtiter plate shaker (rotary) capable of shaking at 300–1000 rpm.
- ❑ Phosphate-buffered saline plus 0.05% Tween-20 for plate washing or MSD Wash Buffer, catalog # R61AA-1
- ❑ Adhesive plate seals
- ❑ Deionized water

[1] SULFO-TAG conjugated detection antibodies should be stored in the dark.

[2] Blockers D–G, D–M, and D–R can tolerate up to 5 freeze–thaw cycles. Alternatively, aliquots of Blockers D–G, D–M, and D–R can be stored at 2–8°C for up to 1 month.

Safety

Use safe laboratory practices and wear gloves, safety glasses, and lab coats when handling kit components. Handle and dispose of all hazardous samples properly in accordance with local, state, and federal guidelines.

Additional product-specific safety information is available in the safety data sheet (SDS), which can be obtained from MSD Customer Service.

Best Practices and Technical Hints

- Do not mix or substitute reagents from different sources or different kit lots.
- Dilute calibrators and samples in polypropylene microcentrifuge tubes; use a fresh pipette tip for each dilution; vortex after each dilution before proceeding.
- Avoid prolonged exposure of detection antibody (stock or diluted) to light. During the antibody incubation step, plates do not need to be shielded from light except for direct sunlight.
- Shaking should be vigorous with a rotary motion between 300 and 1000 rpm.
- Avoid bubbles in wells at all pipetting steps. Bubbles may lead to variable results; bubbles introduced when adding read buffer may interfere with signal detection. Do not shake plate after adding read buffer.
- Use reverse pipetting when necessary to avoid introduction of bubbles, and pipette to the bottom corner of empty wells.
- When using an automated plate washer, rotating the plate 180 degrees between wash steps may improve assay precision.
- Gently tap the plate to remove residual fluid after washing.
- Read buffer should be at room temperature when added to the plate.
- Keeping time intervals consistent between adding read buffer and reading the plate should improve inter-plate precision. Limit the time the plate is incubated with read buffer.
- Remove plate seals prior to reading the plate.
- If an incubation step needs to be extended, avoid letting the plate dry out by keeping sample or detection antibody solution in the wells.
- If assay results are above the top of the calibration curve, dilute samples, and repeat the assay.
- When running partial plates, use the sector map in the instrument or software manual to select the wells to be used. Seal the unused portion of the plate with a plate seal to avoid contaminating unused wells. After reading a partial plate, remove fluid, reseal unused sectors, return plate to its original foil pouch with desiccant pack, and seal pouch with tape. Partially used plates may be stored for up to 14 days at 2–8°C.
- You may adjust volumes proportionally when preparing reagents.

Reagent Preparation

Bring all reagents to room temperature.

Prepare Calibrator Dilutions

MSD supplies calibrator for the Human α-Synuclein Kit at a concentration that is 20-fold higher than the recommended highest standard. We recommend a 7-point calibration curve with 4-fold serial dilution steps and a zero calibrator blank. Thaw the stock calibrator and keep on ice, then add to diluent at room temperature to make the calibration curve solutions.

Calibrator	Human α-Synuclein (pg/mL)	Dilution Factor
Stock Calibrator	200 000	
Calibrator-01	10 000	20
Calibrator-02	2 500	4
Calibrator-03	625	4
Calibrator-04	156	4
Calibrator-05	39	4
Calibrator-06	9.8	4
Calibrator-07	2.4	4
Calibrator-08	0	n/a

To prepare 7 calibration solutions plus a zero calibrator blank for up to 4 replicates:

1. Prepare the highest calibrator by adding 15 µL of stock calibrator to 285 µL of Diluent 35. Mix well.
2. Prepare the next calibrator by transferring 50 µL of the highest calibrator to 150 µL of Diluent 35. Mix well. Repeat 4-fold serial dilution 5 times to generate 7 calibrators.
3. Use Diluent 35 as the blank.

Sample Collection and Handling

CSF

Sample collection methods and pre-analytical conditions may cause variability in measured analyte levels.[2,5,6] MSD recommends reviewing current literature and protocols for collection and handling of CSF samples.

Serum and Plasma

Plasma prepared in heparin tubes commonly displays additional clotting following thawing of the sample. Remove clots and all solid material by centrifugation. Avoid multiple freeze–thaw cycles for serum and plasma samples.

Dilute Samples

Dilute samples with Diluent 35. For human CSF and serum samples, MSD recommends a minimum 8-fold sample dilution; however depending on the sample set under investigation, you may need to use a higher dilution factor. For example, to dilute 8-fold, add 20 µL of sample to 140 µL of Diluent 35.

Prepare Detection Antibody Solution

MSD provides detection antibody as a 50X stock solution. The working solution is 1X. Prepare the detection antibody solution immediately prior to use.

For 1 plate, combine:

- ❑ 60 µL of 50X SULFO-TAG Anti-α-Synuclein Antibody
- ❑ 300 µL of 10% Blocker D-G (1% final concentration in detection antibody solution)
- ❑ 150 µL of 2% Blocker D-M (0.1% final concentration in detection antibody solution)
- ❑ 30 µL of 10% Blocker D-R (0.1% final concentration in detection antibody solution)
- ❑ 2460 µL of Diluent 100

Prepare Read Buffer

MSD provides Read Buffer T as a 4X stock solution. The working solution is 2X.

For 1 plate, combine:

- ❑ 10 mL of Read Buffer T (4X)
- ❑ 10 mL of deionized water

You may keep excess diluted read buffer in a tightly sealed container at room temperature for up to 1 month.

Prepare MSD Plate

MSD plates are pre-coated with capture antibodies (Figure 1) and exposed to a proprietary stabilizing treatment to ensure the integrity and stability of the immobilized antibodies. Plates may be used as delivered; no additional preparation (e.g., pre-wetting) is required.

Protocol

1. **Block plate.** Add 150 µL of Diluent 35 to each well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 1 hour.

   You may prepare calibrators, samples, and detection antibody during incubation.

2. **Wash, Add Detection Antibody Solution and Sample:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 25 µL of detection antibody solution to each well. Add 25 µL of diluted sample or calibrator per well. Seal the plate with an adhesive plate seal and incubate at room temperature with shaking for 2 hours.

   You may prepare diluted read buffer during incubation.

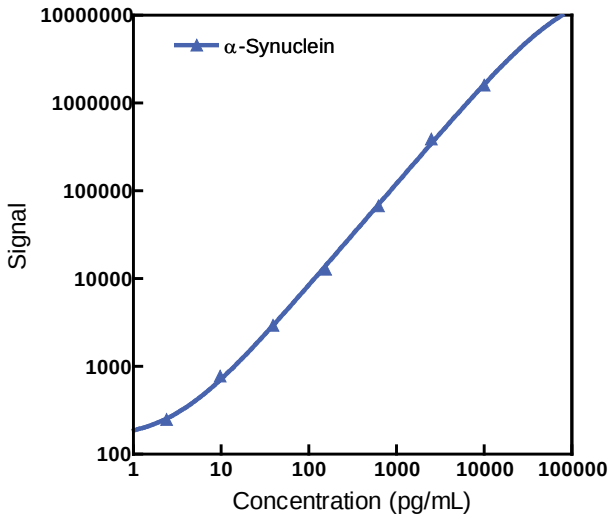
3. **Wash and Read:** Wash the plate 3 times with at least 150 µL/well of PBS-T. Add 150 µL of 2X Read Buffer T to each well. Read plate on the MSD instrument. No incubation in read buffer is required before reading the plate.

Curve Fitting

Run at least 1 set of calibrators in duplicate to generate the calibration curve. The calibration curve is modeled using least squares fitting algorithms so that signals from the calibrators can be used to calculate the concentration of analyte in the samples. The assay has a wide dynamic range (4 logs), which allows for accurate quantification in samples without the need for multiple dilutions or repeated testing The data displayed below were generated by DISCOVERY WORKBENCH® analysis software using a 4-parameter, logistic curve-fitting model (sigmoidal dose-response) with a $1/Y^2$ weighting function, which provides a better fit of data over a wide dynamic range, particularly at the low end of the calibration curve.

Typical Data

The following calibration curve graph illustrates the dynamic range of the assay. Actual signals will vary. Best quantification of unknown samples will be achieved by generating a calibration curve for each plate using a minimum of 2 replicates of calibrators.



α-Synuclein		
Conc. (pg/mL)	Average Signal	%CV
0	90	10.5
2.4	248	2.7
9.8	778	3.1
39	2 945	2.8
156	12 816	1.9
625	67 390	2.7
2 500	388 844	2.8
10 000	1 595 860	2.1

Sensitivity

The lower limit of detection (LLOD) is a calculated concentration corresponding to a signal 2.5 standard deviations above the background (zero calibrator).

	α-Synuclein
Average LLOD (pg/mL)	0.57

Assay Components

Calibrators

The assay calibrator uses recombinant α-Synuclein, (residues 1-140), expressed in *E.coli*.

Antibodies

	Source Species		
Analyte	MSD Capture Antibody	MSD Detection Antibody	Assay Generation
α-Synuclein	Rabbit Monoclonal	Mouse Monoclonal	A

References

1. Stefanis L, et al. α-Synuclein in Parkinson's disease. Cold Spring Harb Perspect Med. 2012;4:a009399.
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3. Trojanowski JQ, et al. Parkinson's disease and related synucleinopathies are a new class of nervous system amyloidoses. Neurotoxicology. 2002;23:457-60.
4. Scherzer CR, et al. GATA transcription factors directly regulate the Parkinson's disease-linked gene α-synuclein. PNAS. 2008;105:10907-12.
5. Mattsson N, et al. Inter-laboratory variation in cerebrospinal fluid biomarkers for Alzheimer's disease: united we stand, divided we fall. Clin Chem Lab Med. 2010;48:603-7.
6. Zetterberg H, et al. Clinical proteomics in neurodegenerative disorders. Acta Neurol Scand. 2008;118:1-11.

Summary Protocol

Human α-Synuclein Kits

MSD provides this summary protocol for your convenience. Please read the entire detailed protocol prior to performing the Human α-Synuclein assays.

Sample and Reagent Preparation

Bring all reagents to room temperature. The calibration curve, diluted samples, and detection antibody solution should be prepared during step 1 and used within one hour of preparation.

Prepare 7 calibration solutions in Diluent 35 using the supplied calibrator:

- Dilute the stock calibrator by adding 15 µL of stock calibrator to 285 µL of Diluent 35. Mix well.
- Perform a series of 4-fold dilution steps and prepare a zero calibrator blank.

Dilute samples 8-fold in Diluent 35 before adding to the plate.

Prepare detection antibody solution by diluting stock detection antibody and blockers in Diluent 100.

Prepare 2X Read Buffer T by diluting stock 4X Read Buffer T 2-fold with deionized water.

Step 1: Block Plate

Add 150 µL/well of Diluent 35.

Incubate at room temperature with shaking for 1 hour.

Step 2: Wash, Add Detection Antibody Solution and Sample

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 25 µL/well of 1X detection antibody solution.

Add 25 µL/well of sample (calibrators or diluted samples).

Incubate at room temperature with shaking for 2 hours.

Step 3: Wash and Read Plate

Wash plate 3 times with at least 150 µL/well of PBS-T.

Add 150 µL/well of 2X Read Buffer T.

Analyze plate on MSD instrument.

Plate Diagram