Human α-Synuclein ELISA Kit

Catalog Number KHB0061 (96 tests)

Pub. No. MAN0014532 **Rev.** 4.0 (30)

CAUTION! This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state, and local regulations for disposal.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Human α-Synuclein ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of human α-synuclein in cell and tissue lysates. The assay will recognize both natural and recombinant human α-synuclein. Although performance characterization of the kit was one primarily on human cell lines, cross-reactivity of the kit with mouse and rat cells was not observed.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KHB0061 (96 tests)
Hu α-Synuclein Standard; contains 0.1% sodium azide.	2 vials
Standard Diluent Buffer; contains 0.1% sodium azide	25 mL
Antibody Coated Plate; 96-well plate	1 plate
Hu α-Synuclein Detection Antibody; contains 0.1% sodium azide, blue dye [1]	6 mL
Anti-Rabbit IgG HRP (100X); contains 3.3 mM thymol	0.125 mL
HRP Diluent; contains 3.3 mM thymol, yellow dye [1]	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Adhesive Plate Covers	3

[1] To avoid pipetting mistakes, colored Detection Antibody and HRP Diluent are provided to monitor the addition of solution to each well. Dyes do not interfere with test results.

Materials required but not supplied

- Distilled or deionized water
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions; beakers, flask and cylinders for preparation of reagents
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Prepare Cell Extraction Buffer

Note: See the *ELISA Technical Guide* for detailed information on preparing Cell Extraction Buffer.

1. Prepare Cell Extraction Buffer.

   Cell Extraction Buffer consists of 10 mM Tris (pH 7.4), 100 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1 mM NaF, 20 mM $Na_4P_2O_7$, 2 mM Na_3VO_4, 1% Triton™ X-100, 10% glycerol, 0.1% SDS, and 0.5% deoxycholate.
2. Immediately before use, add PMSF (0.3 M stock in DMSO) to 1 mM and 50 µL protease inhibitor cocktail (e.g., Sigma Cat. No. P-2714) for each 1 mL of Cell Extraction Buffer.

Prepare cell lysate

1. Collect cells by centrifugation (non-adherent cells) or scraping from culture flasks (adherent cells), then wash cells twice with cold PBS.
2. Remove and discard the supernatant and collect the cell pellet. The pellet can be stored at −80°C and lysed at a later date if desired.
3. Lyse the cell pellet in Cell Extraction Buffer for 30 minutes, on ice. Vortex at 10-minute intervals.

   Note: The volume of Cell Extraction Buffer used depends on the number of cells in the cell pellet, and expression levels of human α-synuclein. Researchers must optimize the extraction procedures for their own applications.
4. Transfer the lysate into microcentrifuge tubes and centrifuge at 13,000 rpm for 10 minutes at 4°C.
5. Transfer the supernatant into clean microcentrifuge tubes. Samples can be stored at −80°C (avoid multiple freeze-thaw cycles).

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

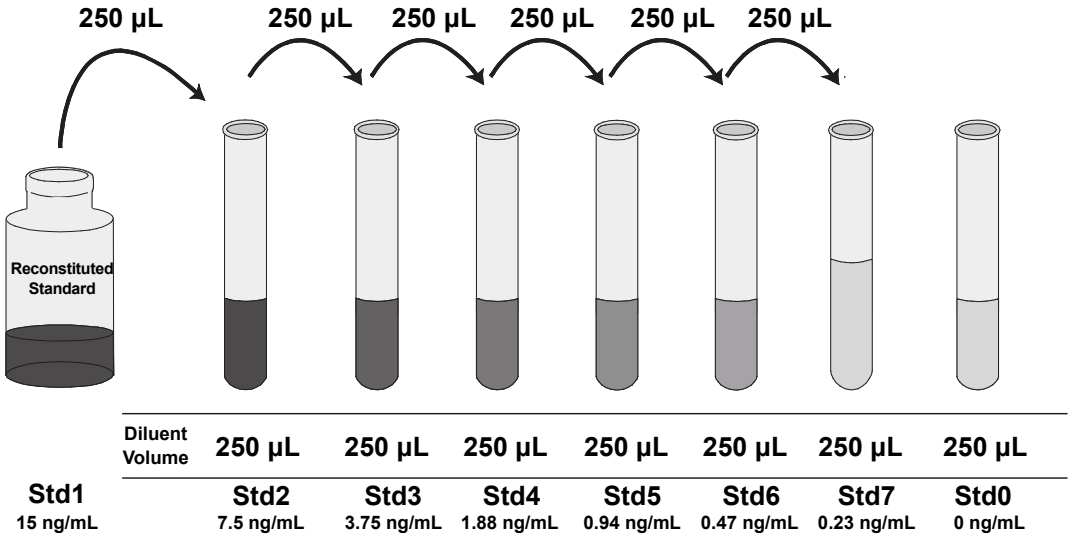
- Dilute samples containing α-Synuclein protein extracted from cells with Standard Diluent Buffer at least 1:5. This dilution is necessary to reduce the matrix effect of the cell lysis buffer. SDS concentration should be less than 0.01% before adding to plate.

Dilute standards

Note: The Hu α-Synuclein Standard is prepared using purified recombinant protein, expressed in *E. coli*.

Note: Use glass or plastic tubes for diluting standards.

1. Reconstitute Hu α-Synuclein Standard to 15 ng/mL with Standard Dilution Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 15 ng/mL human α-synuclein. **Use the standard within 1 hour of reconstitution.**
2. Add 250 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 7.5, 3.75, 1.88, 0.94, 0.47, 0.23, and 0 ng/mL human α-synuclein.
3. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
4. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity. Once thawed, store the Standard Diluent Buffer at 4°C.



Prepare 1X Anti-Rabbit IgG HRP solution

Note: Prepare 1X Anti-Rabbit IgG HRP solution within 15 minutes of usage.

The Anti-Rabbit IgG HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Anti-Rabbit IgG HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of HRP Diluent. Mix thoroughly.
2. Return the unused Anti-Rabbit IgG HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 **Bind antigen and add detector**

a. Add 50 µL of standards, controls, or samples (see "Pre-dilute samples"on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Add 50 µL of Hu α-Synuclein Detection Antibody solution into each well except the chromogen blanks.
c. Tap the side of the plate to mix. Cover the plate with a plate cover and incubate 3 hours at room temperature.
d. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 **Add IgG HRP**

a. Add 100 µL Anti-Rabbit IgG HRP into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 30 min at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 **Add Stabilized Chromogen**

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

4 **Add Stop Solution**

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

The following data was obtained for the various standards over the range of 0 to 15 ng/mL human α-synuclein.

Standard Human α-Synuclein (ng/mL)	Optical Density (450 nm)
15	3.40
7.5	2.70
3.75	1.73
1.88	0.91
0.94	0.48
0.47	0.30
0.23	0.22
0	0.16

Inter-assay precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	45.9	18.9	6.9
Standard Deviation	3.6	1.1	0.4
% Coefficient of Variation	7.9	5.7	5.5

Intra-assay precision

Samples of known human α-synuclein concentration were assayed in replicates of 16 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (ng/mL)	43.69	17.9	6.8
Standard Deviation	3.2	0.8	0.4
% Coefficient of Variation	7.2	4.7	5.2

Cross-reactivity

β-Synuclein was found to have <5% cross-reactivity, while the rest were found to have no cross-reactivity.

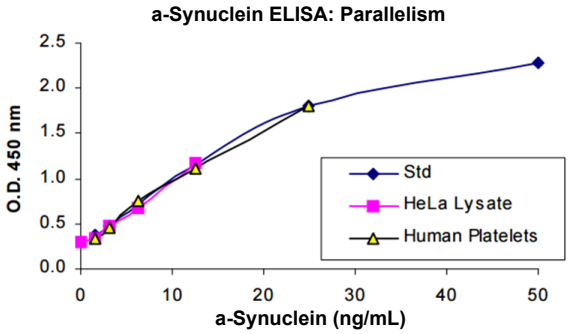
Linearity of dilution

HeLa cell extract (200 µg/mL) was diluted in Standard Diluent Buffer over the range of the assay and measured for human α-synuclein. Linear regression analysis of sample values versus the expected concentration yielded a correlation coefficient of 0.99.

Dilution	Cerebrospinal Fluid		
	Measured (ng/mL)	Expected	
		(ng/mL)	%
Neat	18.99	18.99	100
1/2	7.88	9.49	83
1/4	3.82	4.74	80
1/8	2.04	2.37	85
1/16	0.97	1.18	82
1/32	0.59	0.59	100
1/64	0.28	0.29	97

Parallelism

Natural human α-synuclein from extracts of HeLa cells and human platelets at 10 µg were serially diluted in Standard Diluent Buffer. The optical density of each dilution was plotted against the human α-synuclein standard curve. The standard accurately reflects full length human α-synuclein content in samples.



Recovery

To evaluate recovery, recombinant human α-synuclein at various levels was spiked into the brain tissue extraction buffer, guanidine HCl, human plasma and human cerebral spinal fluid and percent recovery calculated over endogenous levels. On average, the recovery in brain tissue extraction buffer was 110%, the recovery in 50 mM guanidine HCl was 86%, the recovery in plasma was 108%, and the recovery in cerebral spinal fluid was 114%.

Sensitivity

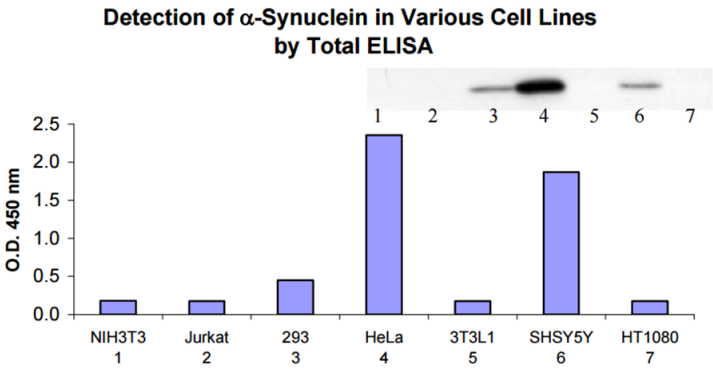
The analytical sensitivity of this assay is 0.2 ng/mL of human α-synuclein as determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

This level of sensitivity is approximately equivalent to the human α-synuclein content of 1,000 HeLa cells. Sensitivity of this ELISA was compared to Western blotting using known quantities of human α-synuclein. Data presented in the figure below show the sensitivity of the ELISA is at least 10X greater than that of western blotting. The bands shown in the Western blotting data were developed using rabbit anti-human α-synuclein and an alkaline phosphatase-conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.

Specificity

The Human α-Synuclein ELISA Kit is specific for measurement of total human α-synuclein protein. The following proteins were tested in the assay: Pras40, Tau, APP, Akt, BNDF, PKC γ, β-amyloid 40 and 42, β-Synuclein.

The expression of human α-synuclein in various cell lines was detected by Human α-Synuclein ELISA Kit . The Human α-Synuclein ELISA Kit is specific for measurement of human α-synuclein, and is consistent with Western blotting (insert). The blot was probed with human α-synuclein rabbit polyclonal antibody and developed using an alkaline phosphatase-conjugated anti-rabbit IgG followed by chemiluminescent substrate and autoradiography.



Limited product warranty

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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

The information in this guide is subject to change without notice.