

Store at  
4°C

# PathScan® Total $\alpha$ -Synuclein Sandwich ELISA Kit



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#54269

New 07/17

For Research Use Only. Not For Use In Diagnostic Procedures.

## Species Cross-Reactivity: M, R

**Description:** The PathScan® Total  $\alpha$ -Synuclein Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of  $\alpha$ -Synuclein protein. An  $\alpha$ -Synuclein rabbit mAb has been coated onto the microwells. After incubation with cell lysates, the  $\alpha$ -Synuclein protein is captured by the coated antibody. Following extensive washing,  $\alpha$ -Synuclein mouse detection mAb is added to detect captured  $\alpha$ -Synuclein protein. Anti-mouse, HRP-linked antibody is then used to recognize the bound detection antibody. HRP substrate, TMB, is added to develop color. The magnitude of absorbance for the developed color is proportional to the quantity of  $\alpha$ -Synuclein protein. Antibodies in the kit are custom formulations specific to the kit.

**Background:**  $\alpha$ -Synuclein is a protein of 140-amino acids expressed abundantly in the brain.  $\alpha$ -Synuclein is also the main component of pathogenic Lewy bodies and Lewy neurites. Research studies have shown that mutations of the  $\alpha$ -synuclein gene are linked to Parkinson's disease (1).

## Background References:

(1) Goldberg, M.S. and Lansbury Jr., P.T. (2000) *Nat. Cell Biol.* 2, 115-119.

**Specificity/Sensitivity:** PathScan® Total  $\alpha$ -Synuclein Sandwich ELISA Kit detects endogenous levels of  $\alpha$ -Synuclein protein in mouse or rat tissues, as shown in Figure 1. The kit sensitivity is shown in Figure 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

| Products Included                                      | Item # | Volume   | Solution Color      | Storage Temp |
|--|--------|----------|---------------------|--------------|
| $\alpha$ -Synuclein Rabbit mAb Coated Microwells*      | 24139  | 96 tests |                     | 4°C          |
| $\alpha$ -Synuclein Mouse Detection mAb                | 98415  | 1 each   | Green (lyophilized) | 4°C          |
| Anti-mouse IgG, HRP-linked Antibody (ELISA Formulated) | 13304  | 1 each   | Red (lyophilized)   | 4°C          |
| Detection Antibody Diluent                             | 13339  | 11 ml    | Green               | 4°C          |
| HRP Diluent  | 13515  | 11 ml    | Red                 | 4°C          |
| TMB Substrate  | 7004   | 11 ml    |                     | 4°C          |
| STOP Solution  | 7002   | 11 ml    |                     | 4°C          |
| Sealing Tape   | 54503  | 2 sheets |                     | 4°C          |
| ELISA Wash Buffer (20X)                                | 9801   | 25 ml    |                     | 4°C          |
| ELISA Sample Diluent                                   | 11083  | 25 ml    | Blue                | 4°C          |
| Cell Lysis Buffer (10X)                                | 9803   | 15 ml    |                     | -20°C        |

\* 12 8-well modules -Each module is designed to break apart for 8 tests.

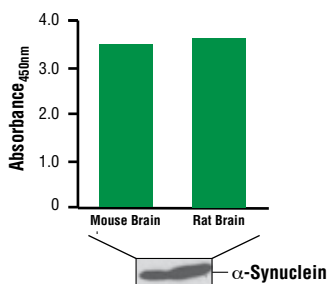


Figure 1:  $\alpha$ -Synuclein protein is detected in mouse or rat brain tissues using the PathScan® Total  $\alpha$ -Synuclein Sandwich ELISA Kit. The absorbance readings at 450 nm are shown in the top figure, while the corresponding western blot using  $\alpha$ -Synuclein (D37A6) XP® Rabbit mAb #4179 is shown in the bottom figure.

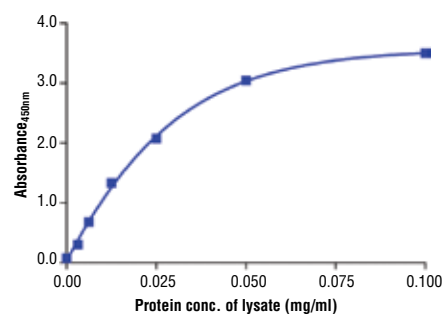


Figure 2: The relationship between protein concentration of lysates from extracts of mouse brain and the absorbance at 450 nm as detected by PathScan® Total  $\alpha$ -Synuclein Sandwich ELISA Kit is shown. Mouse brains were frozen, ground, and then lysed with lysis buffer #9803.

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Applications: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide Species Cross-Reactivity: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

## PathScan® Sandwich ELISA Protocol (for kits with Lyophilized Antibodies)

### A Solutions and Reagents

**NOTE:** Prepare solutions with purified water.

- 1. Microwell strips:** Bring all to room temperature before use.
- 2. Detection Antibody:** Supplied lyophilized as a green colored cake or powder. Add 1.0 ml of Detection Antibody Diluent (green solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted Detection Antibody to 10.0 ml of Detection Antibody Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 3. HRP-Linked Antibody\*:** Supplied lyophilized as a red colored cake or powder. Add 1.0 ml of HRP Diluent (red solution) to yield a concentrated stock solution. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. To make the final working solution, add the full 1.0 ml volume of reconstituted HRP-Linked Antibody to 10.0 ml of HRP Diluent in a clean tube and gently mix. Unused working solution may be stored for 4 weeks at 4°C.
- 4. Detection Antibody Diluent:** Green colored diluent for reconstitution and dilution of the detection antibody (11 ml provided).
- 5. HRP Diluent:** Red colored diluent for reconstitution and dilution of the HRP-Linked Antibody (11 ml provided).
- 6. Sample Diluent:** Blue colored diluent provided for dilution of cell lysates.
- 7. 1X Wash Buffer:** Prepare by diluting 20X Wash Buffer (included in each PathScan® Sandwich ELISA Kit) in purified water.
- 8. Cell Lysis Buffer:** 10X Cell Lysis Buffer #9803: This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: Add 1 mM phenylmethyl-sulfonyl fluoride (PMSF) immediately before use.
- 9. TMB Substrate (#7004).**
- 10. STOP Solution (#7002).**

*\*Note: Some PathScan® ELISA Kits may include HRP-Linked Streptavidin in place of HRP-Linked Antibody.*

### B Preparing Cell Lysates

#### For adherent cells.

1. Aspirate media when the culture reaches 80–90% confluence. Treat cells by adding fresh media containing regulator for desired time.
2. Remove media and rinse cells once with ice-cold 1X PBS.
3. Remove PBS and add 0.5 ml ice-cold 1X Cell Lysis Buffer plus 1 mM PMSF to each plate (10 cm diameter) and incubate the plate on ice for 5 min.
4. Scrape cells off the plate and transfer to an appropriate tube. Keep on ice.
5. Sonicate lysates on ice.
6. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

#### For suspension cells

1. Remove media by low speed centrifugation (~1200 rpm) when the culture reaches 0.5–1.0 x 10<sup>6</sup> viable cells/ml. Treat cells by adding fresh media containing regulator for desired time.
2. Collect cells by low speed centrifugation (~1200 rpm) and wash once with 5–10 ml ice-cold 1X PBS.
3. Cells harvested from 50 ml of growth media can be lysed in 2.0 ml of 1X Cell Lysis Buffer plus 1 mM PMSF.
4. Sonicate lysates on ice.
5. Microcentrifuge for 10 min (14,000 rpm) at 4°C and transfer the supernatant to a new tube. The supernatant is the cell lysate. Store at –80°C in single-use aliquots.

### C Test Procedure

1. After the microwell strips have reached room temperature, break off the required number of microwells. Place the microwells in the strip holder. Unused microwells must be resealed and stored at 4°C immediately.
2. Cell lysates can be undiluted or diluted with Sample Diluent (supplied in each PathScan® Sandwich ELISA Kit, blue color). Individual datasheets for each kit provide a sensitivity curve that serves as a reference for selection of an appropriate starting lysate concentration. The sensitivity curve shows typical kit assay results across a range of lysate concentration points.
3. Add 100 µl of each undiluted or diluted cell lysate to the appropriate well. Seal with tape and press firmly onto top of microwells. Incubate the plate for 2 hr at 37°C. Alternatively, the plate can be incubated overnight at 4°C.
4. Gently remove the tape and wash wells:
  - a. Discard plate contents into a receptacle.
  - b. Wash 4 times with 1X Wash Buffer, 200 µl each time for each well.
  - c. For each wash, strike plates on fresh towels hard enough to remove the residual solution in each well, but do not allow wells to completely dry at any time.
  - d. Clean the underside of all wells with a lint-free tissue.
5. Add 100 µl of reconstituted Detection Antibody (green color) to each well (refer to Section A, Step 2). Seal with tape and incubate the plate at 37°C for 1 hr.
6. Repeat wash procedure (Section C, Step 4).
7. Add 100 µl of reconstituted HRP-Linked secondary antibody (red color) to each well (refer to Section A, Step 3). Seal with tape and incubate the plate for 30 min at 37°C.
8. Repeat wash procedure (Section C, Step 4).
9. Add 100 µl of TMB Substrate to each well. Seal with tape and incubate the plate for 10 min at 37°C or 30 min at 25°C.
10. Add 100 µl of STOP Solution to each well. Shake gently for a few seconds.

**NOTE:** Initial color of positive reaction is blue, which changes to yellow upon addition of STOP Solution.

11. Read results.
  - a. **Visual Determination:** Read within 30 min after adding STOP Solution.
  - b. **Spectrophotometric Determination:** Wipe underside of wells with a lint-free tissue. Read absorbance at 450 nm within 30 min after adding STOP Solution.