Revision 9

#80244 Store at 4°C

PathScan® RP Phospho-Tau (Ser404) Sandwich ELISA Kit

1 Kit (96 assays)

Species Cross Reactivity	UniProt ID:	Entrez-Gene Id:
H M R	#P10636-8	#4137

Cell Signaling TECHNOLOGY®

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Product Includes	Product #	Quantity	Color	Storage Temp
Tau Rabbit mAb Coated Microwells	24240	96 tests		4°C
Phospho-Tau (Ser404) Rabbit Detection mAb	43162	1 ea	Red (Lyophilized)	4°C
HRP Diluent	13515	5.5 ml	Red	4°C
TMB Substrate	7004	11 ml		4°C
STOP Solution	7002	11 ml		4°C
Sealing Tape	54503	2 ea		4°C
ELISA Wash Buffer (20X)	9801	25 ml		4°C
Cell Lysis Buffer (10X)	9803	15 ml		-20°C

*The microwell plate is supplied as 12 8-well modules - Each module is designed to break apart for 8 tests.

Description

The rapid protocol (RP) PathScan® RP Phospho-Tau (Ser404) Sandwich ELISA Kit is a solid phase sandwich enzyme-linked immunosorbent assay (ELISA) that detects endogenous levels of tau protein phosphorylated at Ser404 in a reduced assay time of 1.5 hours. Incubation of cell lysates and detection antibody on the coated microwell plate forms a sandwich with tau protein phosphorylated at Ser404 in a single step. The plate is then extensively washed and TMB reagent is added for signal development. The magnitude of absorbance for the developed color is proportional to the quantity of tau protein phosphorylated at Ser404. Learn more about all of your ELISA kit options here.

*Antibodies in this kit are custom formulations specific to kit.

Specificity/Sensitivity

The PathScan® RP Phospho-Tau (Ser404) Sandwich ELISA Kit detects endogenous levels of tau protein phosphorylated at Ser404. The kit sensitivity is shown in Figures 1 and 2. This kit detects proteins from the indicated species, as determined through in-house testing, but may also detect homologous proteins from other species.

Background

Tau is a heterogeneous microtubule-associated protein that promotes and stabilizes microtubule assembly, especially in axons. Six isoforms with different amino-terminal inserts and different numbers of tandem repeats near the carboxy terminus have been identified, and tau is hyperphosphorylated at approximately 25 sites by Erk, glycogen synthase kinase-3 (GSK-3), and CDK5 (1,2). Phosphorylation decreases the ability of tau to bind to microtubules. Neurofibrillary tangles are a major hallmark of Alzheimer's disease (AD); these tangles are bundles of paired helical filaments (PHFs) composed of hyperphosphorylated tau. In particular, phosphorylation at Ser396 by GSK-3 or CDK5 destabilizes microtubules. Furthermore, research studies have shown that inclusions of tau are found in a number of other neurodegenerative diseases, collectively known as tauopathies (1,3).

Investigators have shown that tau phosphorylation at Ser404 destabilizes microtubules and that tau is hyperphosphorylated at Ser404 in Alzheimer's disease (2,4-6).

Background References

1. Johnson, G.V. and Stoothoff, W.H. (2004) *J Cell Sci* 117, 5721-9.
2. Hanger, D.P. et al. (1998) *J Neurochem* 71, 2465-76.
3. Bramblett, G.T. et al. (1993) *Neuron* 10, 1089-99.
4. Shiurba, R.A. et al. (1996) *Brain Res* 737, 119-32.
5. Evans, D.B. et al. (2000) *J Biol Chem* 275, 24977-83.
6. Bertrand, J. et al. (2010) *Neuroscience* 168, 323-34.

Cross-Reactivity Key

H: human **M:** mouse **R:** rat **Hm:** hamster **Mk:** monkey **Vir:** virus **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
GP: Guinea Pig **Rab:** rabbit **All:** all species expected

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#80244

PathScan® RP Phospho-Tau (Ser404) Sandwich ELISA Kit

PathScan® Sandwich ELISA Protocol (Rapid Protocol)

NOTE: This protocol is for PathScan® kits that use an HRP directly conjugated to the detection antibody **(Rapid Protocol)**, rather than a 2-step method where the detection antibody and a secondary-HRP are added sequentially.

A. Solutions and Reagents

NOTE: Prepare solutions with deionized/purified water or equivalent.

1. **Microwell strips:** Bring all to room temperature before opening bag/use. Unused microwell strips should be returned to the original re-sealable bag containing the desiccant pack and stored at 4°C.
2. **Detection Antibody:** Reconstitute lyophilized Detection Antibody (red colored cake) with 5.5 mL HRP Diluent. Incubate at room temperature for 5 min with occasional gentle mixing to fully reconstitute. For best results, use immediately following antibody reconstitution. Unused reconstituted Detection Antibody may be stored for up to 4 weeks at 4°C, although there may be some loss of signal compared to freshly reconstituted antibody.
3. **HRP Diluent:** Red colored diluent for reconstitution and dilution of the Detection Antibody that is linked to HRP.
4. **1X ELISA Wash Buffer:** Prepare by diluting ELISA Wash Buffer (20X) (included in each kit) to 1X with deionized water.
5. **1X Cell Lysis Buffer:** Prepare by diluting 10X Cell Lysis Buffer #9803 to 1X with deionized water. This buffer can be stored at 4°C for short-term use (1–2 weeks). Recommended: When using to prepare cell lysates, add Protease/Phosphatase Inhibitor Cocktail (#5872, not supplied) and 1 mM phenylmethyl- sulfonyl fluoride (PMSF, #8553, not supplied) immediately before use.
6. **TMB Substrate (#7004):** Bring to room temperature before use.
7. **STOP Solution (#7002):** Bring to room temperature before use.

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 including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail 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 10^6 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 including 1 mM PMSF and Protease/Phosphatase Inhibitor Cocktail.
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

NOTE: Equilibrate all materials and prepared reagents to room temperature prior to running the assay.

1. Prepare all reagents as indicated above (Section A).
2. Samples should be undiluted or diluted with 1X Cell Lysis Buffer to a 2X protein concentration in order to achieve a final 1X protein concentration upon addition of the Detection Antibody. 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 results across a range of lysate concentration points.
3. Add 50 µL of each sample to the appropriate wells.
4. Add 50 µL of the Detection Antibody to each well.
5. Seal the plate and incubate for 1 hour at room temperature on a plate shaker set to 400 rpm (moderate agitation).
6. 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.
7. Add 100 µL of TMB Substrate to each well. Seal with tape and incubate the plate in the dark for 15 min at room temperature on a plate shaker (400 rpm, moderate agitation) or alternatively for 10 min at 37°C without shaking.
8. 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.

9. 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.

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