Store at -20°C

#8648

Parkinson's Research Antibody Sampler Kit

☑ 1 Kit
(5 x 20 µl)

Cell Signaling TECHNOLOGY®

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
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info@cellsignal.com
Web ■ www.cellsignal.com

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

Products Included	Product #	Quantity	Mol. Wt.	Isotype
DJ-1 (D29E5) XP® Rabbit mAb	5933	20 µl	22 kDa	Rabbit IgG
LRRK2 (D18E12) Rabbit mAb	13046	20 µl	290 kDa	Rabbit IgG
Parkin (Prk8) Mouse mAb	4211	20 µl	50 kDa	Mouse IgG2b
PINK1 (D8G3) Rabbit mAb	6946	20 µl	60, 50 kDa	Rabbit IgG
α-Synuclein (D37A6) XP® Rabbit mAb	4179	20 µl	18 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat
Anti-mouse IgG, HRP-linked Antibody	7076	100 µl		Horse

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol, and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:
Western blotting 1:1000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Parkinson's Research Antibody Sampler Kit provides an economical means of detecting target proteins related to Parkinson's disease. The kit contains enough primary and secondary antibody to perform two western blots per primary.

Background: Parkinson's disease (PD), the second most common neurodegenerative disease after Alzheimer's, is a progressive movement disorder characterized by rigidity, tremors, and postural instability. The pathological hallmark of PD is progressive loss of dopaminergic neurons in the substantia nigra of the ventral midbrain and the presence of intracellular Lewy bodies in surviving neurons of the brain stem (1). Research studies have shown that various genes and loci (α-synuclein/PARK1 and 4, parkin/PARK2, UCH-L1/PARK5, PINK1/PARK6, DJ-1/PARK7, LRRK2/PARK8, synphilin-1, and NR4A2) are genetically linked to PD (2).

α-Synuclein, a 140 amino acid protein expressed abundantly in the brain, is a major component of aggregates found in Lewy bodies (3). Parkin is involved in protein degradation through the ubiquitin-proteasome pathway, and investigators have shown that mutations in Parkin cause early onset of PD (4). In the case of autosomal recessive juvenile Parkinsonism (AR-JP), deletions have been found on chromosome 6 in the Parkin gene (5). PTEN induced putative kinase 1 (PINK1) is a mitochondrial serine/threonine kinase involved in the normal function and integrity of mitochondria, as well as a reduction of cytochrome c release from mitochondria (6-8). PINK1 phosphorylates Parkin and promotes its translocation to mitochondria (7). Mutations of PINK1 are associated with loss of protective function, mitrochondrial dysfunction, aggregation of α-synuclein, and proteasome dysfunction (6,8). DJ-1 is involved in multiple cellular functions; it has been shown to cooperate with Ras to increase cell transformation, to regulate transcription of the androgen receptor, and may function as an indicator of oxidative stress, while loss-of-function mutations in DJ-1 cause early onset of PD (9-12). Dopamine D2 receptor-mediated functions are greatly impaired in DJ-1 (-/-) mice, resulting in reduced long-term depression (13). Leucine-rich repeat kinase 2 (LRRK2) contains amino-terminal leucine-rich repeats (LRR), a Ras-like small GTP binding protein-like (ROC) domain, an MLK protein kinase domain, and a carboxy-terminal WD40-repeat. At least 20 LRRK2 mutations have been linked to PD (14). The most prevalent mutation, G2019S, causes increased LRRK2 kinase activity, leading to progressive neurite loss and decreased neuronal survival (15).

Specificity/Sensitivity: DJ-1 (D29E5) XP® Rabbit mAb, LRRK2 (D18E12) Rabbit mAb, Parkin (Prk8) Mouse mAb, and PINK1 (D8G3) Rabbit mAb recognize endogenous levels of respective target proteins. α-Synuclein (D37A6) XP® Rabbit mAb recognizes endogenous levels of the α isoform of synuclein protein.

Source/Purification: Monoclonal antibodies are produced by immuninzing animals with a recombinant protein specific to the carboxy terminus of Parkin protein, a synthetic peptide corresponding to residues surrounding Lys148 of human DJ-1 protein, a synthetic peptide corresponding to residues surrounding Pro2080 of human LRRK2 protein, a synthetic peptide corresponding to residues surrounding Pro140 of human PINK1 protein, or a synthetic peptide corresponding to residues surrounding Glu105 of mouse α-synuclein protein.

Background References:

(1) Fahn, S. (2003) *Ann N Y Acad Sci* 991, 1-14.
(2) Moore, D.J. et al. (2005) *Annu Rev Neurosci* 28, 57-87.
(3) Goldberg, M.S. and Lansbury, P.T. (2000) *Nat Cell Biol* 2, E115-9.
(4) Borrelli, E. (2005) *Neuron* 45, 479-81.
(5) Polymeropoulos, M.H. et al. (1997) *Science* 276, 2045-7.
(6) Liu, W. et al. (2009) *PLoS One* 4, e4597.
(7) Kim, Y. et al. (2008) *Biochem Biophys Res Commun* 377, 975-80.
(8) Petit, A. et al. (2005) *J Biol Chem* 280, 34025-32.
(9) Bonifati, V. et al. (2003) *Science* 299, 256-9.
(10) Nagakubo, D. et al. (1997) *Biochem Biophys Res Commun* 231, 509-13.
(11) Takahashi, K. et al. (2001) *J Biol Chem* 276, 37556-63.
(12) Mitsumoto, A. and Nakagawa, Y. (2001) *Free Radic Res* 35, 885-93.
(13) Goldberg, M.S. et al. (2005) *Neuron* 45, 489-96.
(14) Mata, I.F. et al. (2006) *Trends Neurosci* 29, 286-93.
(15) MacLeod, D. et al. (2006) *Neuron* 52, 587-93.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **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.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.

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Western Immunoblotting Protocol (Primary Antibody Incubation in Milk)

For Western blots, incubate membrane with diluted antibody in 5% w/v nonfat dry milk, 1X TBS, 0.1% Tween-20 at 4°C with gentle shaking, overnight.

A Solutions and Reagents

NOTE: Prepare solutions with Milli-Q or equivalently purified water.

1. 1X Phosphate Buffered Saline (PBS)
2. **1X SDS Sample Buffer:** 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% w/v bromophenol blue or phenol red
3. **Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
4. **10X Tris Buffered Saline (TBS):** To prepare 1 liter of 10X TBS: 24.2 g Tris base, 80 g NaCl; adjust pH to 7.6 with HCl (use at 1X).
5. Nonfat Dry Milk (weight to volume [w/v])
6. **Blocking Buffer:** 1X TBS, 0.1% Tween-20 with 5% w/v nonfat dry milk; for 150 ml, add 15 ml 10X TBS to 135 ml water, mix. Add 7.5 g nonfat dry milk and mix well. While stirring, add 0.15 ml Tween-20 (100%).
7. **Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
8. **Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% nonfat dry milk; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g nonfat dry milk and mix well. While stirring, add 20 µl Tween-20 (100%).
9. **Phototope®-HRP Western Blot Detection System** #7072**:** Includes biotinylated protein ladder, secondary anti-mouse (#7076) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO® chemiluminescent reagent and peroxide.
10. Prestained Protein Marker, Broad Range (Premixed Format) #7720
11. Biotinylated Protein Ladder Detection Pack #7727
12. **Blotting Membrane:** This protocol has been optimized for nitrocellulose membranes, which CST recommends. PVDF membranes may also be used.

B Protein Blotting

A general protocol for sample preparation is described below.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl per plate of 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
5. Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
6. Microcentrifuge for 5 minutes.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

NOTE: CST recommends loading prestained molecular weight marker (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights.

8. Electrotransfer to nitrocellulose or PVDF membrane.

C Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
3. Wash three times for 5 minutes each with 15 ml of TBS/T.
4. Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
5. Wash three times for 5 minutes each with 15 ml of TBS/T.
6. Incubate membrane with HRP-conjugated secondary antibody (1:2000) and HRP-conjugated anti-biotin antibody (1:1000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hour at room temperature.
7. Wash three times for 5 minutes each with 15 ml of TBS/T.

D Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO®, 0.5 ml 20X Peroxide and 9.0 ml Milli-Q water) with gentle agitation for 1 minute at room temperature.

NOTE: LumiGLO® substrate can be further diluted if signal response is too fast.

2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10-second exposure should indicate the proper exposure time.

NOTE: Due to the kinetics of the detection reaction, signal is most intense immediately following LumiGLO® incubation and declines over the following 2 hours.