

#2567 Store at -20°C

# LRRK2 Antibody

✓ 100 µl  
(10 Western mini-blot)

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

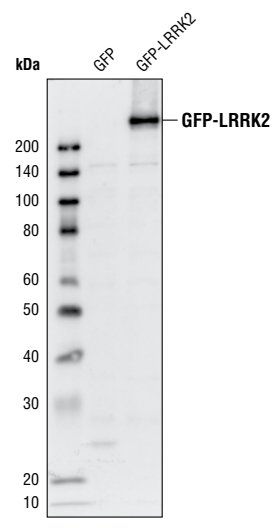
Applications	Species Cross-Reactivity	Molecular Wt.	Source
W	H	290 kDa	Rabbit

**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 (protein aggregates of  $\alpha$ -synuclein, ubiquitin and other components) in surviving neurons of the brain stem (1). Various genes and loci ( $\alpha$ -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).

Leucine-rich repeat kinase 2 (LRRK2) contains amino-terminal leucine-rich repeats (LRR), a Ras-like small GTP binding protein-like (ROC) domain, a MLK protein kinase domain and carboxy-terminal WD40-repeat. At least 20 LRRK2 mutations have been linked to PD, the G2019S mutation being the most prevalent (3). The G2019S mutation causes increased LRRK2 kinase activity, which induces a progressive reduction in neurite length, leading to progressive neurite loss and decreases neuronal survival (4). The MLK inhibitor CEP-1347 is explored in PD clinical trials indicating that LRRK2 may have value as a therapeutic target for treatment of PD (5).

**Specificity/Sensitivity:** The LRRK2 Antibody is recommended for transfected total LRRK2 only. The LRRK2 antibody detects a background band in extracts of wild-type and LRRK2 knock-out mouse brain at ~290kD.

**Source/Purification:** Polyclonal antibodies are produced by immunizing rabbits with a synthetic peptide (KLH-coupled) surrounding Gly2090 of human LRRK2. Antibodies are purified by protein A and peptide affinity chromatography.



Western blot analysis of extracts from HEK-293 cells, transfected with GFP alone (lane 1) or GFP-LRRK2 (lane 2), using LRRK2 Antibody.

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

**Recommended Antibody Dilutions:**  
Western blotting 1:1000

- Companion Products:**
- $\alpha$ -Synuclein (IF Preferred) Antibody #2628
  - $\alpha$ -Synuclein Antibody #2642
  - $\alpha$ -Synuclein (Syn204) Mouse mAb #2647
  - $\alpha$ / $\beta$ -Synuclein (Syn205) Mouse mAb #2644
  - p35 Antibody #2673
  - Parkin (Park8) Mouse mAb #4211
  - Parkin Antibody #2132
  - DJ-1 Antibody #2134
  - Phospho-Tyrosine Hydroxylase (Ser40) Antibody #2791
  - Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071
  - Anti-rabbit IgG, HRP-linked Antibody #7074
  - Prestained Protein Marker, Broad Range (Premixed Format) #7720
  - Biotinylated Protein Ladder Detection Pack #7727
  - 20X LumiGLO® Reagent and 20X Peroxide #7003

- Background References:**
- (1) Fahn, S. (2003) *Ann. NY Acad. Sci.* 991, 1–14.
  - (2) Moore, D.J. et al. (2005) *Annu. Rev. Neurosci.* 28, 57–87.
  - (3) Mata, I.F. et al. (2006) *Trends Neurosci.* 29, 286–293.
  - (4) MacLeod, D. et al. (2006) *Neuron* 52, 587–593.
  - (5) Parkinson Study Group. (2004) *Neurology* 62, 330–332.

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

## Western Immunoblotting Protocol (Primary Antibody Incubation in BSA)

For Western blots, incubate membrane with diluted antibody in 5% w/v BSA, 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.

- 1X Phosphate Buffered Saline (PBS)
- 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
- Transfer Buffer:** 25 mM Tris base, 0.2 M glycine, 20% methanol (pH 8.5)
- 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).
- Nonfat Dry Milk (weight to volume [w/v])
- 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%).
- Wash Buffer:** 1X TBS, 0.1% Tween-20 (TBS/T)
- Bovine Serum Albumin (BSA)
- Primary Antibody Dilution Buffer:** 1X TBS, 0.1% Tween-20 with 5% BSA; for 20 ml, add 2 ml 10X TBS to 18 ml water, mix. Add 1.0 g BSA and mix well. While stirring, add 20 µl Tween-20 (100%).
- Phototope<sup>®</sup>-HRP Western Blot Detection System #7071:** Includes biotinylated protein ladder, secondary anti-rabbit (#7074) antibody conjugated to horseradish peroxidase (HRP), anti-biotin antibody conjugated to HRP, LumiGLO<sup>®</sup> chemiluminescent reagent and peroxide.
- Prestained Protein Marker, Broad Range (Premixed Format) #7720
- Biotinylated Protein Ladder Detection Pack #7727
- 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.

- Treat cells by adding fresh media containing regulator for desired time.
- Aspirate media from cultures; wash cells with 1X PBS; aspirate.
- 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.
- Sonicate for 10–15 seconds to shear DNA and reduce sample viscosity.
- Heat a 20 µl sample to 95–100°C for 5 minutes; cool on ice.
- Microcentrifuge for 5 minutes.
- Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm).

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

- Electrotransfer to nitrocellulose or PVDF membrane.

### C Membrane Blocking and Antibody Incubations

**NOTE:** Volumes are for 10 cm x 10 cm (100 cm<sup>2</sup>) of membrane; for different sized membranes, adjust volumes accordingly.

- (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 minutes at room temperature.
- Incubate membrane in 25 ml of blocking buffer for 1 hour at room temperature.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- Incubate membrane and primary antibody (at the appropriate dilution) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
- Wash three times for 5 minutes each with 15 ml of TBS/T.
- 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.
- Wash three times for 5 minutes each with 15 ml of TBS/T.

### D Detection of Proteins

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

**NOTE:** LumiGLO<sup>®</sup> substrate can be further diluted if signal response is too fast.

- 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<sup>®</sup> incubation and declines over the following 2 hours.