NEUROMICS

PTEN-induced Kinase/PINK1 Data Sheet

Catalog Number:	RA19013	**Host:**	Rabbit
Product Type:	Affinity Purified Antibody	**Species Reactivity:**	Human, Mouse
Immunogen Sequence:	Reacts with residues 258-274 (YRKSKRGPKQLAPHPNI) of human PINK1.	**Format:**	Liquid. 100 ug in 100 ul (1 mg/ml) in PBS containing 0.02% sodium azide
Applications:	Western blotting 1:500 – 1:1000 Immunohistochemistry 1:1000		
Storage:	Dilutions listed as a recommendation. Optimal dilution should be determined by investigator. Maintain at +2-8°C for 3 months or at -20°C for longer periods. Stable for 1 year. *Avoid repeated freeze-thaw cycles.*		
References:	Micha M.M. Wilhelmus, Susanne M.A. van der Pol, Quentin Jansen, Maarten E. Witte, Paul van der Valk, Annemieke J.M. Rozemuller, Benjamin Drukarch, Helga E. de Vries and Jack Van Horssen. Association of Parkinson disease-related protein PINK1 with Alzheimer disease and multiple sclerosis brain lesions. Free Radical Biology and Medicine. Volume 50, Issue 3, 1 February 2011, Pages 469-476. Alberto Ferri, Paolo Fiorenzo, Monica Nencini, Mauro Cozzolino, Maria Grazia Pesaresi, Cristiana Valle, Sara Sepe, Sandra Moreno, and Maria Teresa Carrì Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. Hum. Mol. Genet., first published on Sep 20, 2010 as doi: doi:10.1093/hmg/ddq383.		

Application Notes

Immunostaining Tissue

Solutions

PBS - sodium phosphate-buffered (100 mM; pH 7.2) isotonic (0.9% NaCl, w/v) saline Antibody dilution buffer (PBS with 0.1% non-ionic detergent, such as Triton X-100 or Tween-20). For anti-fading, use Neuromics' i-BRITE Plus –Catalog#: SF40000 or make your won fluorescein anti-fading reagent -- Make up a 2 mg/ml phenylene diamine solution in PBS (phenylene diamine requires extensive vortexing to put it into solution). Once the phenylene diamine is completely dissolved, add an equal volume of glycerol and mix. This reagent will last about a week at -20^{O}C. Discard this reagent when it starts to turn dark brown.

Other Reagents

Fluorescein-labeled goat anti-rabbit IgG

1. Prepare your tissue sections or cultured cells as you normally would. Wash your sections or cells for 1 min with PBS at room temperature.
2. Incubate your sections or cells with your chicken primary antibodies (diluted in "antibody dilution buffer") for at least 1 hour at room temperature. The concentration of your antibody may be anywhere from 1:50-1:150 depending on the titre of the antibody and the concentration of your antigen.
3. Wash your sections or cells over a 10 minute period at room temperature (with two changes of PBS).
4. Incubate your sections or cells with fluorescein-labeled goat anti-chicken IgG (1:500 dilution in "antibody dilution buffer" for 1 hour at room temperature. Be sure to keep these slides or culture dishes in subdued light (e.g., in a drawer) to avoid bleaching of the fluorescein dye.
5. Repeat step #4
6. Add a drop of "fluorescence anti-fading reagent" (i-BRITE Plus) to your sections or cells. Place a coverslip over the section. If you want to reduce messiness, you may also seal the coverslip by painting the edges with nail polish.
7. Store the slides or culture dishes in the refrigerator (in the dark).

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Western Blotting

1. Run gel as usual. Take gel out of electrophoresis apparatus. Cut into segments as required; Part of gel can be stained directly in Coomassie brilliant blue R-250 (2.5 g Coomassie Brilliant Blue R-250, 450 mls methanol, 100 mls glacial acetic acid, water to 1 liter). Part to be used for electroblotting is put into tap water on shaker, after first having marked it unambiguously to identify top/bottom, left and right etc.
2. Leave in water on shaker for 5 minutes. This step can be substituted by washing the gel in electro-transfer buffer (see below) for 5 minutes.
3. We use a semidry blotter, which we have found to be quicker, more economical and easier than fully submerged blotting methods. We cut Whatman 3M filter papers to the size of our gels, and place three of these onto the semi dry blotter. These are then wet with transfer buffer (we routinely use 3.03 g Tris base, 14.4 g Glycine, 10% Methanol per liter). The gel is put onto the filters and a prewetted nitrocellulose filter is put ontop of the gel. Alternately put a PVDF membrane on top; if you are using PVDF remember it is essential to prewet the PVDF in 100% methanol. Great care should be taken to ensure that no air bubbles are anywhere in this stack of membranes. Then three more wetted Whatman 3M filters should be placed ontop of the pile, again taking great care not to have any bubbles in pile. Put the top onto the apparatus and screw it down. Proteins in transfer buffer are negative in charge mostly due to residual SDS and they therefore move from -ve to +ve pole. So the +ve electrode is above the nitrocellulose and the -ve side is below the gel.
4. Run for 30 minutes to 1 hour at ~100mA. The most reliable way of doing this is to use a powerful power supply 200-500mA and put it on constant voltage, with a setting of 5 to 10 Volts. Low molecular weight proteins (20kDa or less) will transer in 30 minutes at 5 Volts, while higher molecular weight (150kDa or more) transfer in 60 minutes at 10 Volts.
5. After running disassemble the apparatus and remove nitrocellulose filter. Stain this for 5 minutes on shaker in Ponceau reagent (0.25% Ponceau S in 40% methanol and 15% acetic acid). Destain with regular SDS-PAGE gel destain solution (7.5% methanol, 10% acetic acid). If you transferred efficiently, the proteins can be seen as pale pink bands. This tells you whether the transfer was O.K. or not and also exactly where the bands are. You can photograph, photocopy or mark the position of the bands directly with a pencil. If you can't see any bands at this stage, it's probably smart to try to optimize steps 3 and 4. The gel may be discarded or may be stained as usual in coomassie, to see how much protein is left behind.
6. After Ponceau staining put the nitrocellulose filter into blocking solution, such as 1% bovine serum albumin (BSA) or 1% Carnation non fat milk (NFM), for 20 minutes to 1 hr at RT or 37°C. Since the NFM works just as well as BSA but is much cheaper, there is really no good reason to use BSA. Ponceau staining will fade to become completely invisible. Carry on with antibody incubations etc.

Antibody Incubations:

1. Put in antibody solutions. Volume should be enough to cover blot and allow it to float freely when you agitate. In initial experiments, antibody concentration should generally be about 1:100 - 1:1,000 for ascites, CL350 tissue culture supernatant or antiserum, undiluted to 1:10 for monoclonal supernatant, and about 1-10µg/ml for a pure IgG. If dilution brings antibody concentration to less than 50 µgs/ml, add some BSA or NFM to act as carrier protein (e.g. make BSA or NFM concentration 1mg/ml). Incubate for at least 1 hour with shaking (can be room temperature or at 37°C, can also do overnight at 4°C).
2. Wash membranes in TBS (10mM Tris, 154mM NaCl, pH=7.5 plus 0.1% Tween 20) for 3 times at least five minutes each time with extensive agitation.
3. Incubate in second antibody (peroxidase-conjugate, phosphatase conjugate or radioactive). Add BSA or NFM carrier as before if necessary. Incubate for at least one hour at room temperature or 37°C can also do overnight at 4°C with shaking as before.
4. Wash membranes in TBS (10mM Tris, 154mM NaCl, pH=7.5 plus 0.1% Tween 20) for 3 times at least five minutes each time with extensive agitation.

A. Alkaline Phosphatase Blot System

1. Incubate in alkaline phosphatase conjugated antibody against the primary antibody (e.g. Goat anti-mouse, rabbit or chicken; buy from Sigma or some other trusted source). Typical concentration is 1:1,000 in TBS (10mM Tris/HCl, 154mM NaCl, pH=7.5). Add a small amount of BSA or NFM to act as carrier. Incubate for 1 hour at room temperature (or 37°C) with shaking.
2. Wash in TBS three times 5 minutes each. (N.B. the alkaline phosphatase enzyme is inhibited by EDTA, which chelates zinc and magnesium, and by phosphate, which inhibits forward reaction. Make sure therefore you use TBS which is EDTA and phosphate free- Don't make up developer in PBS!)
3. Put into developer. Buffer is 100mM Tris/HCl, 100mM NaCl, 5mM MgCl2 pH=9.5. To 10ml of this add 33µl BCIP-T (5-bromo-4-chloro-3-indolyl phosphate, p-toluidine salt, make up 50mg/ml in water or Dimethyl formamide; in water makes a yellow suspension) and 33µl of NBT (Nitro Blue Tetrazolium, also 50mg/ml in water). Can store these solutions at -20°C. Can buy this solution made up already from Sigma. Reaction product is purple, and appears in a few minutes; can incubate for up to an hour if the signal is weak. Watch development of reaction and stop with water. Some of background disappears on drying.

B. Horse Radish Peroxidase Staining

After washing of blots in TBS or PBS (must not have azide in wash buffer! This inhibits the peroxidase enzyme) add reaction mixture. This is; 20 mls 0.1M Tris/HCl pH=7.2 (Vecta stain buffer). 200 µl NiCl (80 mg/ml), 6 µl 30% hydrogen peroxide, 1ml of 5mgs/ml diaminobenzidine. (Wear gloves, DAB is carcinogenic). Alternate protocol; Make 20 mls ammonium acetate buffer (50mM, pH=5.0). Add 1 ml of 10mg/ml Diaminobenzidine, 40µl 30% hydrogen peroxide. Brown reaction product is seen in 1-10 minutes, not quite so nice as above method.

C. Chemiluminescence Staining

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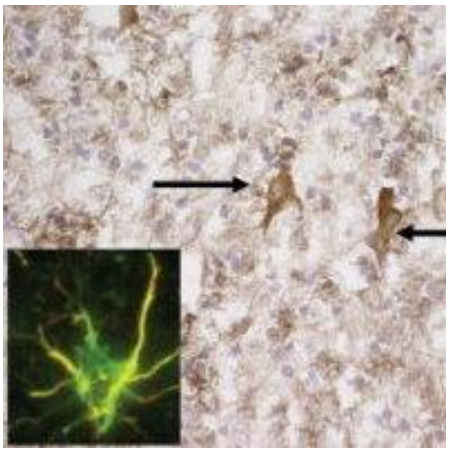
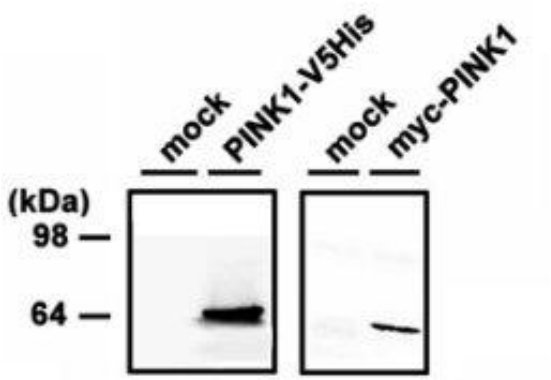
Chemiluminescence has an advantage of perhaps an order of magnitude greater sensitivity than the dye based methods above. In addition, several films may be exposed from a single blot, giving an advantage in interpretation of weak and strong signals on the same membrane. However it requires a darkroom to perform and is more expensive in reagents. Reagents are generally bought in a kit, and we recommend simply following the kit instructions.

Image: In active lesions PINK1 immunostaining was intense in reactive astrocytes (arrows). Double labeling of PINK1 (green) with the astrocytic marker GFAP (red) demonstrated PINK1 expression in astrocytes (inset).



Note: Jack Van Horssen et al. ,using this PINK1, showed IHC staining of astrocytes in temporal neocortical tisue harvested from AD and MD patients postmortem (in AD,PINK1 was found to colocalize with classic senile plaques and vascular amyloid depositions). see Free Radical Biology and Medicine. Volume 50, Issue 3, 1 February 2011, Pages 469-476.



Western blot of C-terminally V5His-tagged human PINK1 or N-terminally myc-tagged human PINK1 expressed in HEK293T cells. The PINK1 antibody at 1:1,000 dilution detects transfected PINK1 protein.

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Related Antibodies

Name	Catalog #	Type	Species	Applications	Size	Price
14-3-3 eta	MO22126	Mouse IgG	H; M; R	IF; WB	100 ul	$245
4-Hydroxynonenal (HNE)	GT19004	Goat IgG	H	WB	100 ul	$295
8-Hydroxydeoxyguanosine (8OHdG)	GT19001	Goat IgG	B; Ca; H; M; P; Pr; R	IHC	100 ul	$295
BDNF	CH15000	Chicken IgY	H; R	IHC; WB; E	100 ug	$365
BDNF	MO15115	Mouse IgG	H	IHC; WB; E	500 ug	$325
Calbindin	MO20016	Mouse IgG	H	IHC	100 ug	$175
Calmodulin	MO20017	Mouse IgG	H	IHC	100 ug	$175
CaMKII	RA18006	Rabbit IgG	H; M; Pr; R	WB	100 ul	$350
Caspase-3	GT15044	Goat IgG	H	WB; IP	100 ug	$345
Caspase-3, active	RA15046	Rabbit IgG	H; M	ICC; IHC	50 ug	$255
Caspase-9	GT15045	Goat IgG	H	ICC; IHC; WB	50 ul 100 ul	$190 $345
Caspase-10/b-Flice 2	RA15047	Rabbit IgG	H; M	WB	100 ug	$275
Caspase-12	RA15048	Rabbit IgG	M; R	WB	100 ug	$315
Cathepsin B (Human)	GT15046	Goat IgG	H	WB; E	100 ug	$365
Cathepsin B (Mouse)	GT15047	Goat IgG	M	IHC; WB; E	100 ug	$365
Cathepsin D	GT15042	Goat IgG	M	IHC; WB; IP	100 ug	$365
Cathepsin F	MO15096	Mouse IgG	H	IHC; WB; IP; E	500 ug	$325
Cathepsin G	MO20021	Mouse IgG	H	IHC; WB	100 ul	$125
Cathepsin L (Human)	GT15048	Goat IgG	H	IHC; WB; E	50 ug 100 ug	$89 $345

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Name	Catalog #	Type	Species	Applications	Size	Price
Cathepsin O	GT15197	Goat IgG	H	IHC; WB; IP; E	100 ug	$365
Cathepsin S	GT15198	Goat IgG	H	IHC; WB; IP; E	100 ug	$365
Cathepsin V	GT15199	Goat IgG	H	IHC; WB; E	100 ug	$365
DOPA decarboxylase	MO15100	Mouse IgG	H; R	IHC; WB; E	100 ug	$255
DOPA decarboxylase	RA25065	Mouse IgG	R	WB	100 ul	$325
Dopamine beta-Hydroxylase	RA24600	Rabbit IgG	B; M; R	IF; IHC; WB	100 ul	$365
Dopamine beta-Hydroxylase	SO25002	Sheep IgG	H; M; Pr	WB	30 ug	$325
Doublecortin/DCX	MO22113	Mouse IgG	B; H; M; P; R	IF; WB	100 ul	$245
GDNF Biotinylated	GT15007B	Goat IgG	H; R	IHC; WB	50 ug	$385
GDNF Receptor Alpha 1	MO15093	Mouse IgG	R	IHC; WB; E	500 ug	$325
GDNF Receptor Alpha 1	GT15108	Goat IgG	H	IHC; WB; E	100 ug	$345
GDNF Receptor Alpha 1	GT15004	Goat IgG	H; M; R	ICC; IHC; WB; E	100 ug	$365
GDNF Receptor Alpha 1 Biotinylated	GT15004B	Goat IgG	H; R	IHC; WB	50 ug	$385
GDNF Receptor Alpha 2	GT15005	Goat IgG	H; M; R	ICC; IHC; WB; E	100 ug	$365
GDNF Receptor Alpha 2 Biotinylated	GT15005B	Goat IgG	H; R	IHC; WB	50 ug	$385
GDNF Receptor Alpha 4	GT15083	Goat IgG	M	IHC; WB; E	100 ug	$365
Glutamine Synthetase	RA25062	Rabbit IgG	B; H; M; R	IHC; WB	100 ul	$285
Mitofusion 2/MFN2	RA26001	Rabbit IgG	H; M; R; Rb	IHC; WB	100 ul	$375
NMDA Receptor 1, N1	RA25036	Rabbit IgG	R	IHC; WB	25 ug	$285
NMDA NR1 Pan	MO25041	Rabbit IgG	R	WB	15 ug	$285
NMDA Receptor 2A	RA25037	Rabbit IgG	H; R	IHC; WB; IP	10 ug	$285

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Name	Catalog #	Type	Species	Applications	Size	Price
phospho-NMDA Receptor 1 (Ser890)	RA18011	Rabbit IgG	H; M; R	WB	100 ul	$370
PARK2 Co-regulated/PACRG	RA30023	Rabbit IgG	H; R	IF; IHC; WB; E	50 ug	$425
PARK7/DJ-1	RA19006	Rabbit IgG	H; M; R	IHC; WB	100 ul	$250
Parkin	RA18014	Rabbit IgG	H; M; R	ICC; WB; IP	100 ul	$370
Parkin-2	GT15043	Goat IgG	H	IHC; WB; E	100 ug 50 ug	$365 $89
PMP22	MO25033	Mouse IgG	H; Pr	IHC	100 ul	$285
Po (P-Zero)	CH23009	Chicken IgY	H; M	ICC; IHC; WB	100 ul	$99
PTEN-induced kinase/PINK1	RA19013	Rabbit IgG	H; M	IHC; WB	100 ul	$275
Peripherin	CH22111	Chicken IgY	Ca; H; M; R	IHC	100 ul	$245
Peripherin	CH23016	Chicken IgY	H; M	IHC	200 ul	$250
Peripherin	RA22109	Rabbit IgG	Ca; H; M; R	ICC; WB	100 ul	$245
Persephin	MO15086	Mouse IgG	H	IHC; WB	100 ug	$215
Peripherin	MO22106	Mouse IgG	Ca; H; M; R	ICC; IF; WB	500 ul	$225
Presenilin 1	RA18020	Rabbit IgG	H; M; Pr; R	WB; IP	200 ul	$330
Presenilin 1	GT15027	Goat IgG	H	IHC; WB; E	100 ug	$365
Presenilin 2	RA18004	Rabbit IgG	H; M; Pr; R	ICC; IHC; WB; IP	100 ul	$310
Synaptophysin	MO20000	Mouse IgG	H; R	IHC; WB	100 ul	$175
Synaptosomal Associated Protein 25/SNAP25	RA30045	Rabbit IgG	M; R	IHC; WB; E	50 ug	$425
alpha Synuclein	MO22111	Mouse IgG	H; M; R	IF; WB	100 ul	$295
alpha Synuclein	MO22112	Mouse IgG	H; M; R	IF; WB	100 ul	$275

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Name	Catalog #	Type	Species	Applications	Size	Price
alpha-Synuclein	GT15112	Goat IgG	H	IHC; WB; E	100 ug	$365
alpha-Synuclein	RA18021	Rabbit IgG	H; M; Pr; R	WB; IP	100 ul	$330
Tyrosine Hydroxylase	CH23006	Chicken IgY	H; M	ICC; IHC; WB	100 ul	$99
Tyrosine Hydroxylase	MO20001	Mouse IgG	H; M; R	ICC; WB	100 ul	$200
Tyrosine Hydroxylase	SO25000	Sheep IgG	H; M; Pr; R	ICC; IF; IHC; WB	100 ul	$325
phospho-Tyrosine Hydroxylase (Ser40)	RA18026	Rabbit IgG	R	IF; IHC; WB; IP	100 ul	$335
UCHL1	MO22109	Mouse IgG	B; H; R	IF; WB	100 ul	$295
UCHL1	MO25040	Mouse IgG	B; H; R	IF; WB	500 ul	$285
Ubiquitin	MO19005	Mouse IgG	H	IHC; WB	50 ul	$155
Ubiquitin	MO18001	Mouse IgG	H; M; Pr; R	IHC; WB	100 ul	$310
Ubiquitin+1	RA15043	Rabbit IgG	H	IHC; WB	100 ug	$365
Ubiquitin+1	MO15045	Mouse IgG	H	WB	50 ug 100 ug	$115 $205

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