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Datasheet for 600-401-433

Huntington phospho S421 Antibody

Overview

Description:	Anti-Huntington pS421 (RABBIT) Antibody - 600-401-433
Item No.:	600-401-433
Size:	100 µg
Applications:	ELISA, IHC, WB
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	Huntingtin (also known as Huntington's disease protein, Htt and HD protein) is the protein product of a disease gene linked to Huntington's disease, a neuro-degenerative disorder characterized by loss of striatal neurons. This may be caused by an expanded, unstable trinucleotide repeat in the huntingtin gene, which translates as a polyglutamine repeat in the protein product (see partial protein sequence below). The huntingtin gene locus is large, spanning 180 kb and consisting of 67 exons. It is expressed as 2 alternatively polyadenylated forms displaying different relative abundance in various fetal and adult tissues. The genetic defect leading to Huntington's disease may not necessarily eliminate transcription, but may confer a new property on the mRNA or alter the function of the protein. One candidate is the huntingtin-associated protein-1, highly expressed in brain, which has increased affinity for huntingtin protein with expanded polyglutamine repeats. Normal huntingtin protein shows a cytoplasmic localization. This protein is widely expressed with the highest level of expression in the brain (nerve fibers, varicosities, and nerve endings). In the brain, the regions where it can be mainly found are the cerebellar cortex, the neocortex, the striatum, and the hippocampal formation.
Synonyms:	rabbit anti-Huntington pS421 antibody, rabbit anti-Huntingtin pS421 antibody, HD protein, HTT, Huntington Disease Protein, Huntington disease, IT15, LOMARS
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HTT
Reactivity:	Human
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	Huntingtin pS421 Antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to an internal region near aa 400-425 of Human Huntington Disease Protein.
Purity/Specificity:	Anti-Huntingtin pS421 is an affinity purified antibody produced by immunoaffinity chromatography using phospho peptide coupled to agarose beads followed by solid phase adsorption(s) against non-phospho peptide and non-specific peptide to remove any unwanted reactivities. This antibody is specific for phosphorylated human Huntington protein at the pS421 residue. BLAST analysis indicates 100 % homology of the immunizing sequence with Huntington homologues from chimpanzee, pig and chicken. Cross reactivity with Huntington protein homologues from mouse and rat may also occur as sequence homology varies by one amino acid residue in this sequence. Reactivity with Huntington protein from other sources is not known. Minimal reactivity is expected with the non-phosphorylated form of the protein.
Relevant Links:	• UniProtKB - P42858 • NCBI - NP_002102.4 • GeneID - 3064

Application Details

Tested Applications:	ELISA, IHC, WB
Application Note:	Anti-Huntingtin pS421 antibody has been tested for use in ELISA, immunohistochemistry, and by western blot. Specific conditions for reactivity should be optimized by the end user. Expect bands at approximately 350 kDa and 200 kDa in size corresponding to full-length Huntingtin protein and truncated (hypothetical) Huntingtin protein, respectively, by western blotting in the appropriate cell lysate or extract. This antibody is specific for the phosphorylated form of Huntingtin protein at the pS421 residue. The identity of lower molecular bands ~130 kDa is not known.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:10,000 - 1:40,000
IHC:	1:50 - 1:100
WB:	1:500 - 1:3,000

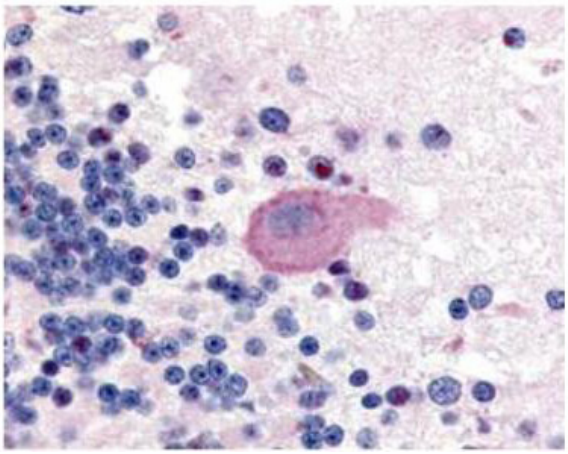
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.96 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

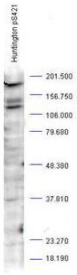
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

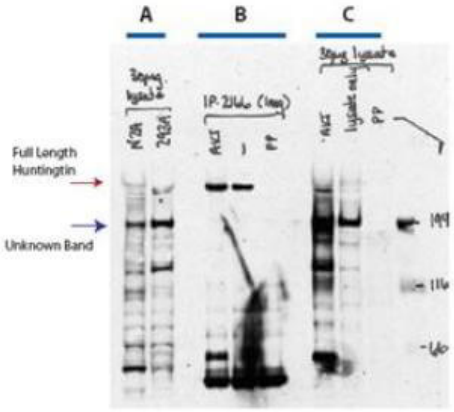
Images



Immunohistochemistry
Rockland's Affinity Purified anti-Huntingtin pS421 antibody was used at a 1:100 dilution to detect phosphorylated Huntingtin by immunohistochemistry in human brain cerebellum. Positive cytoplasmic staining is observed in neurons. Tissue was formalin-fixed and paraffin embedded. Personal Communication, Alan Yen, LifeSpanBiosciences, Seattle, WA.



Western Blot
Western blot analysis is shown using Rockland's Affinity Purified anti-Huntingtin pS421 antibody to detect endogenous protein present in an unstimulated human PC-3 whole cell lysate. Comparison to a molecular weight marker indicates a band of ~190 kDa corresponding to truncated human Huntingtin protein. The blot was incubated with a 1:1,000 dilution of the antibody at room temperature followed by detection using standard techniques. Personal communication, Steven Pelech, Kinexus Inc., Vancouver, BC.



Western Blot
Western blot analysis after AKT and phosphatase treatment is shown using Rockland's Affinity Purified anti-Huntingtin pS421 antibody. In A) untreated lysates from N2A and 293A cells were stained directly using anti-Huntingtin pS421 antibody. Full length staining of Huntingtin is noted, albeit at low levels of expression, as well as a strongly staining band at 200 kDa that may represent staining of truncated protein. In B) staining is shown after immunoprecipitation using a monoclonal antibody (Mab2166) followed by AKT treatment (to phosphorylate), untreated, and phosphatase (PP) treated (dephosphorylate) immunoprecipitated HTT. Full length phosphorylated huntingtin is clearly detected in these immunopurified samples (except dephosphorylated). In C) lysates are treated directly with AKT or PP to alter the phosphorylation status of HTT. Personal communication, Simon Warby, CMMT, Vancouver, BC.

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