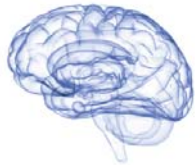


FocusOn No.132



Antibodies for Huntington's Disease

Huntington's disease (HD) is a fatal neurodegenerative disorder which usually occurs in mid-age. It is characterized by psychiatric disorders, involuntary movements and dementia, leading to death within 10-20 years.

Huntingtin is a 350 kDa protein that is altered in HD. The expanded trinucleotide CAG repeat of the huntingtin gene encodes an abnormally expanded polyglutamine stretch in the N-terminus of the protein. The abnormal form of huntingtin aggregates in vitro and forms neuronal intranuclear and cytoplasmic inclusions in HD patients. Furthermore, the expanded polyglutamine repeats have been proposed to cause neuronal degeneration in HD through abnormal interactions with other proteins containing short polyglutamine tracts such as the CREB binding protein (CBP). CREP promotes cell survival and is a major mediator of survival signals in mature neurons.

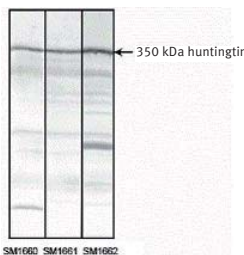


Fig. 1

Fig. 1: Total protein extract of normal human cerebral cortex separated as a strip on SDS-PAGE gel and Western blotted using huntingtin antibodies Cat.-No. **SM1660**, **SM1661** and **SM1662** (from left to right)

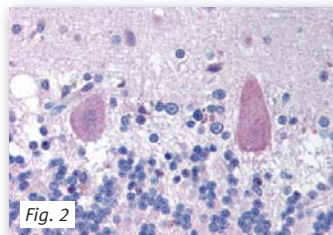


Fig. 2

Fig. 2: Formalin-fixed, paraffin-embedded (FFPE) human brain cerebellum stained with huntingtin antibody Cat.-No. **AP07574PU-N**

Huntingtin-interacting proteins

HIP1 (Huntingtin-interacting protein 1) was identified as a protein that associates with huntingtin. Binding of HIP1 to huntingtin is dramatically reduced following polyglutamine expansion, strongly implicating this interaction in the disease process.

Huntingtin has also been shown to interact with the following proteins: **GAPDH**, **HAP1**, **HIP7 (Huntingtin-interacting protein 7, Optineurin)** and **HIP2 (Huntingtin-interacting protein 2)**.

HIP2 is a ubiquitin conjugating enzyme which binds selectively to a large region at the N-terminus of huntingtin. HIP2-driven ubiquitination of huntingtin marks it for selective degradation via the proteasomal pathway. HIP2 may mediate foam cell formation by the suppression of apoptosis.

HIP9 (Huntingtin-interacting protein 9) is the alpha subunit of the adaptor protein complex 2 (AP-2) which participates in membrane traffic pathways. AP-2 plays a key role in clathrin-dependent endocytosis. Cargo proteins are ferried into clathrin-coated vesicles (CCVs) which fuse with the early endosome. HIP9 (AP-2 alpha subunit) is responsible for orienting AP-2 on the membrane (binding accessible polyphosphoinositide-containing lipids) and also recognizing and binding to the endocytosis signal motif [ED]-X-X-X-L-[LI] of endocytic transmembrane accessory proteins via its C-terminal cytosolic tail.

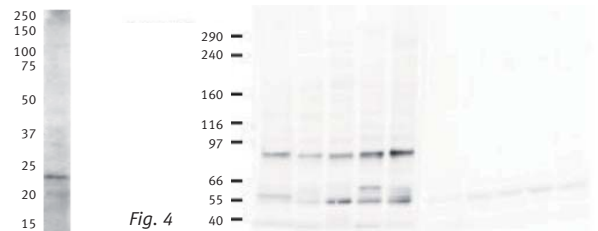


Fig. 3

Fig. 4

Fig. 3: HIP2 antibody Cat.-No. **AP16095PU-N** staining of Jurkat lysate (35 µg total protein per lane)

Fig 4: Western blot using HIP9/AP2A antibody Cat.-No. **AP09207PU-N** shows detection of a band corresponding to human AP2A1 in a various preparations. Lane 1: HeLa nuclear extract; Lane 2: HeLa; Lane 3: 293; Lane 4: A431 and Lane 5: Jurkat whole cell lysates. In lanes 6-10 the antibody was preincubated with 1 µg/ml of the immunizing peptide which effectively blocks the specific reactivity of this antibody with AP2A.

HIP14 is a novel huntingtin-interacting protein. Its interaction with huntingtin is inversely correlated to the polyglutamine length of huntingtin. HIP14 protein, which is enriched in the brain, has been reported to show partial co-localization with huntingtin in the striatum. It is found in a subset of neurons affected in HD. One related protein is **HIP14-like (HIP14L)**, which has 69 % homology to HIP14.



Fig. 5: HIP14/ZDHHC17 antibody Cat.-No. **AP16315PU-N** staining of mouse brain lysate (35 µg protein in RIPA buffer)

Huntingtin-associated protein-1 (HAP1) is highly expressed in brain and has been demonstrated to mediate the neuropathology of HD. HAP1 interacts with huntingtin, with two cytoskeletal proteins (dynactin and pericentriolar autoantigen protein 1) and with a hepatocyte growth factor-regulated tyrosine kinase substrate. The interactions with cytoskeletal proteins and a kinase substrate indicate a role for HAP1 in vesicular trafficking or organelle transport.

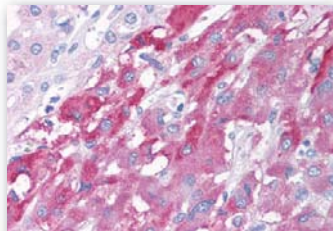


Fig. 6: HAP1 antibody Cat.-No. AP07921PU-N staining of formalin-fixed, paraffin-embedded human adrenal tissue

Key References

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Please refer also to other FocusOns about Neurosciences

FocusOn 130: Antibodies for Alzheimer's Disease
 FocusOn 131: Antibodies for Parkinson's Disease
 FocusOn 133: Antibodies for Amyotrophic Lateral Sclerosis

Name	Property	Host	Clone	Reactivity	Application	Catalog-No.
GAPDH		Mouse	6C5	Hu, Broad	E, IF, WB	ACR001P
GAPDH		Rabbit		Hu, Broad	E, IF, P, WB	AP09372PU-N
GAPDH		Mouse	FF26A/F9	Hu	IP, P, WB	AM01308PU-N
Huntingtin	pSer421	Rabbit		Hu, Ms, Rt	E, P, WB	AP07574PU-N
Huntingtin		Mouse	HDA3E10	Hu, Ms, Rb	C, IP, WB	SM1660
Huntingtin		Mouse	HDB4E10	Hu, Ms, Rb	C, IP, WB	SM1661
Huntingtin		Mouse	HDC8A4	Hu, Ms, Rb	C, IP, WB	SM1662
HIP1		Mouse	4B10	Hu, Rt	WB	AM20001SU-N
HIP1		Mouse	1B11	Hu	WB	AM20002SU-N
HIP2		Rabbit		Hu, Ms	E, WB	AP11971PU-N
Hip2	C-term	Goat		Bov, Can, Hu, Ms, Rt	WB	AP16095PU-N
HIP7/Optineurin	575-591	Rabbit		Hu, Ms, Rt	P, WB	AP08244PU-N
HIP7/Optineurin	115-130	Rabbit		Hu, Ms, Por, Rt	P, WB	AP08487PU-N
HIP7/Optineurin	194-568	Rabbit		Hu	IF, WB	AP20023PU-N
HIP9	3-14	Goat		Can, Chk, Hu, Mky, Ms, Rt	E, WB	AP09207PU-N
HIP9	Center	Rabbit		Hu	E, WB	AP17885PU-N
HIP14	C-term	Goat		Can, Chimp, Hu, Ms, Rt	WB	AP16315PU-N
HIP14	1-246	Rabbit		Can, Hu, Ms, Rt, Zebrafish	WB	AP20024PU-N
HIP14L		Goat		Hu, Ms, Rt	WB	AP16323PU-N
HAP1		Rabbit		Hu, Ms, Rt	WB	AP05702PU-N
HAP1		Rabbit		Hu, Ms, Rt	P, WB	AP07303PU-N
HAP1	639-653	Rabbit		Hu	E, P	AP07921PU-N
HAP1		Goat		Hu	E, WB	AP17012PU-N

Bov: Bovine, Can: Canine, Chk: Chicken, Hu: Human, Mky: Monkey, Ms: Mouse, Rb: Rabbit, Rt: Rat

C: Immunohistochemistry on frozen sections, E: ELISA, IF: Immunofluorescence, IP: Immunoprecipitation, P: Immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections, WB: Western blot