

JPH (Junctophilin) Detection Set

CATALOG No.: PSI-1828

BACKGROUND:

Junctional complexes between the plasma membrane (PM) and endoplasmic/sarcoplasmic reticulum (ER/SR) are a common feature of all excitable cell types and mediate cross talk between cell surface and intracellular ion channels. Junctophilins (JPs) are important components of the junctional complexes. JPs are composed of a carboxy-terminal hydrophobic segment spanning the ER/SR membrane and a remaining cytoplasmic domain that shows specific affinity for the PM (1). Four JPs have been identified as tissue-specific subtypes derived from different genes: JPH1 is expressed in skeletal muscle, JPH2 is detected throughout all muscle cell types, and JPH3 and JPH4 are predominantly expressed in the brain and contribute to the subsurface cistern formation in neurons (1-3). JPH1 is essential for stabilizing the T-tubule and SR membranes to form junctions and provide an environment for the assembly of receptors such as the ryanodine receptor type 1 (RyR1) (4). JPH2-null mice died of embryonic cardiac arrest and human patients with mutations in the JPH2 gene showed hypertrophic cardiomyopathy, demonstrating the importance of this protein (5,6). Mice lacking both JPH3 and JPH4 subtypes exhibit serious symptoms such as impaired learning and memory and are accompanied by abnormal nervous functions (7). A repeat expansion in JPH3 is associated with Huntington disease-like 2 (8). Multiple isoforms of the JPH proteins are known to exist.

KIT CONTENTS:

JPH1 antibody, **Catalog No. 4917 (50µg)**.

JPH2 antibody (CT2), **Catalog No. 4929 (50µg)**.

JPH3 antibody (CT), **Catalog No. 4921 (50µg)**.

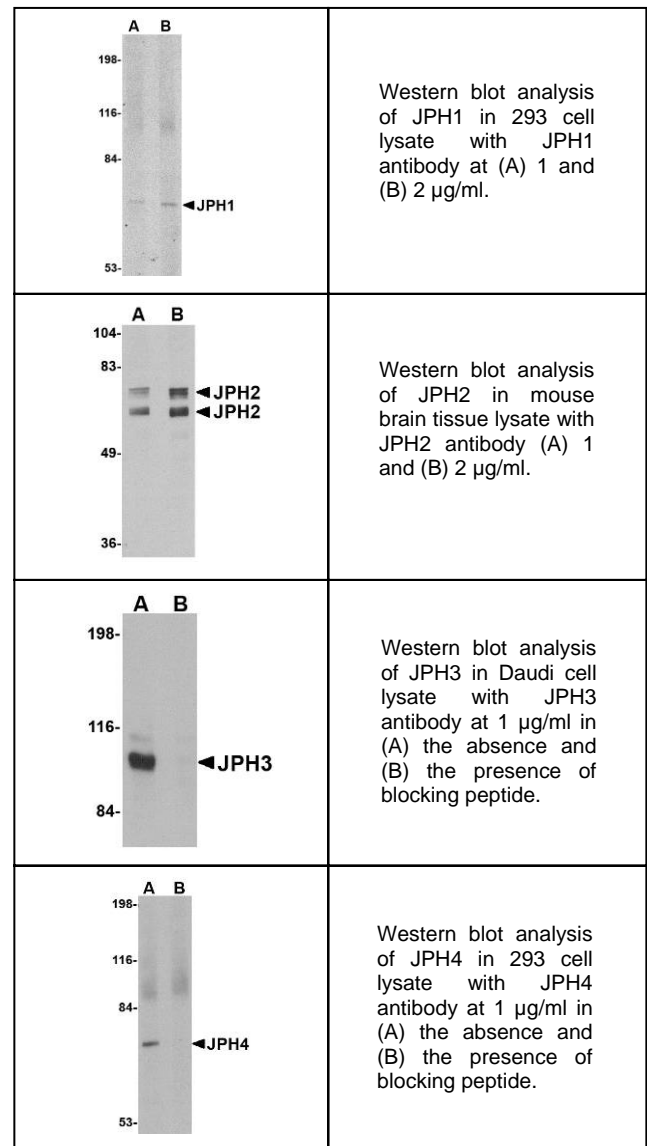
JPH4 antibody (CT), **Catalog No. 4923 (50µg)**.

SOURCE:

Rabbit polyclonal antibodies were raised against peptides corresponding to amino acid sequences from each of the corresponding proteins.

STORAGE:

Antibodies are supplied as affinity chromatography purified IgG in PBS containing 0.02% sodium azide. Antibodies should be stored at -20°C.



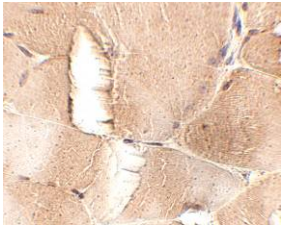
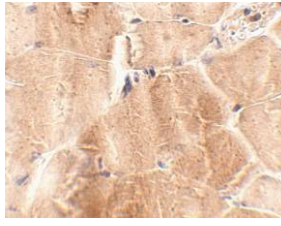
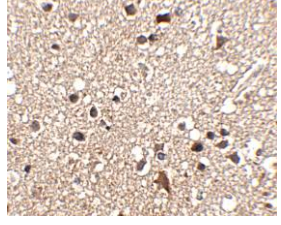
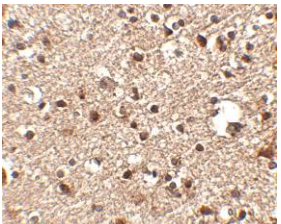
RELATED PRODUCTS:

JPH1 peptide, **Catalog No. 4917P**.

JPH2 peptide (CT2), **Catalog No. 4929P**.

JPH3 peptide (CT), **Catalog No. 4921P**.

JPH4 peptide (CT), **Catalog No. 4923P**.

	Immunohistochemistry of JPH1 in mouse skeletal muscle tissue with JPH1 antibody at 2.5 µg/ml.
	Immunohistochemistry of JPH2 in mouse skeletal muscle tissue with JPH2 antibody at 2.5 µg/ml.
	Immunohistochemistry of JPH3 in human brain tissue with JPH3 antibody at 2.5 µg/ml.
	Immunohistochemistry of JPH4 in human brain tissue with JPH4 antibody at 2.5 µg/ml.

REFERENCES:

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2. Kakizawa S, Kishimoto Y, Hashimoto K, et al. Junctophilin-mediated channel crosstalk essential for cerebellar synaptic plasticity. *EMBO J.* 2007; 26:1924-33.
3. Nishi M, Sakagami H, Komazaki S, et al. Coexpression of junctophilin type 3 and type 4 in brain. *Brain Res. Mol. Brain Res.* 2003; 118:102-10.
4. Phimister AJ, Lango J, Lee EH, et al. Conformation-dependent stability of Junctophilin 1 (JP1) and Ryanodine Receptor type 1 (RyR1) channel complex is mediated by their hyper-reactive thiols. *J. Biol. Chem.* 2007; 282:8867-77.
5. Matsushita Y, Furukawa T, Kasanuki H, et al. Mutation of junctophilin type 2 associated with hypertrophic cardiomyopathy. *J. Hum. Genet.* 2007; 52:543-8.
6. Landstrom AP, Weisleder N, Batalden KB, et al. Mutations in JPH2-encoded junctophilin-2 associated with hypertrophic cardiomyopathy in humans. *J. Mol. Cell Cardiol.* 2007; 42:1026-35.
7. Moriguchi S, Nishi M, Komazaki S, et al. Functional uncoupling between Ca²⁺ release and afterhyperpolarization in mutant hippocampal neurons lacking junctophilins. *Proc. Natl. Acad. Sci.* 2006; 103:10811-6.
8. Holmes SE, O'Hearn E, Rosenblatt A, et al. A repeat expansion in the gene encoding junctophilin-3 is associated with Huntington disease-like 2. *Nat Genet.* 2001; 29:377-8.

APPLICATION:

These polyclonal antibodies can be used for detection of JPH1 – 4 by immunoblot at 1 – 2 µg/ml, and for detection of JPH1 – 4 by immunohistochemistry at 2.5 - 5 µg/ml. **For research use only.**

POSITIVE CONTROLS:

JPH1 antibody: 293 Cell Lysate, **Catalog No. 1210.**

JPH2 antibody: Mouse Brain Tissue Lysate,

Catalog No. 1403.

JPH3 antibody: Daudi Cell Lysate, **Catalog No. 1224.**

JPH4 antibody: 293 Cell Lysate, **Catalog No. 1210.**



WESTERN BLOT PROTOCOL:

- 1) Load 20 to 25 micrograms of whole cell lysate per lane in an SDS-PAGE mini gel.
- 2) Run at 20 mA per gel until the dye front is close to the bottom.
- 3) Transfer the proteins to a nitrocellulose membrane at 250 mA in transfer buffer for 1-4 h, depending on the size of the target protein.
- 4) Incubate the blot with blocking buffer (5% non-fat dry milk in TBS) overnight at 4°C or 2 hr at room temperature (RT).
- 5) Incubate the blot with primary antibody (diluted 1:250 to 1:1000 in blocking buffer) for 1 hr in blocking buffer at RT.
- 6) Wash the blot 3 x 10 min in washing buffer (TBS containing 0.1% Tween 20) with shaking.
- 7) Incubate blot with anti-rabbit IgG-HRP conjugate (diluted 1:10,000 -1:20,000 in blocking buffer) for 1 h in blocking buffer at RT.
- 8) Wash 3 x 10 min in washing buffer with shaking.
- 9) Drain washing buffer, add ECL solution and develop for 1 min.
- 10) Expose to X-ray film for 1 to 30 min.

MATERIALS NEEDED:

Nitrocellulose membrane
Non-fat dry milk
Tween-20
Antibody detection kit

TBS:

- 125 mM NaCl
- 25 mM Tris pH 8.0
- 0.1% Tween 20

SDS/Running Buffer:

- 25 mM Tris
- 192 mM Glycine
- 0.1% SDS

Transfer Buffer:

- 20 mM Tris
- 150 mM Glycine
- 20% methanol
- 0.038% SDS