

Anti-VRK1 (human) antibody, mouse monoclonal (5D1)

71-600 100 μg

Shipping and Storage: Ship at 4°C or -20°C and store at -20°C

Immunogen: Synthetic peptide corresponding to N-terminus of human VRK1,

MPRVKAAQAGRQSSAKRHL-C

Form: 1 mg/ml in PBS- with 50% glycerol, filter-sterilized.

Purity: Mouse monoclonal antibody (5D1) produced in serum-free medium and purified by propriety chromatography under mild conditions (90~98% pure).

Isotype: IgG1 κ

Reactivity: Human VRK1 protein. Not tested with other species.

Applications

- 1. Western blotting (1/200~1/1,000 dilution). Use of highly sensitive chemiluminescence reagents such as Lumi-Light Plus (Roche) or ImmunoStar®LD (Wako, Tokyo) are recommended.
- 2. Immunoprecipitation (assay dependent)
- 3. Immunofluorescence staining (1/100 dilution)
- 4. Immunohistochemistry (assay dependent)
- 5. ELISA (assay dependent)

Background: The VRK1 gene encodes serine/threonine kinase VRK1 (Vaccinia-Related Kinase 1; 396 aa, 45.5 kDa) which is involved in Golgi disassembly during the cell cycle following phosphorylation by PLK3 during mitosis, and required to induce Golgi fragmentation. It acts by mediating phosphorylation of a downstream target protein 'Thr-18' of p53/TP53 and may thereby prevent the interaction between p53/TP53 and MDM2. It also phosphorylates casein and histone H3. Phosphorylation of the BANF1 gene product disrupts its ability to bind DNA, reduces its binding to LEM domain-containing proteins and causes its relocalization from the nucleus to the cytoplasm.

Involvement in disease Defects in VRK1 are the cause of pontocerebellar hypoplasia type 1A (PCH1A); also called pontocerebellar hypoplasia with infantile spinal muscular atrophy or pontocerebellar hypoplasia with anterior horn cell disease. PCH1A is characterized by an abnormally small cerebellum and brainstem, central and peripheral motor dysfunction from birth, gliosis and anterior horn cell degeneration resembling infantile spinal muscular atrophy

Database links: SwissProt: Q99986 Human, Entrez Gene: 7443 Human

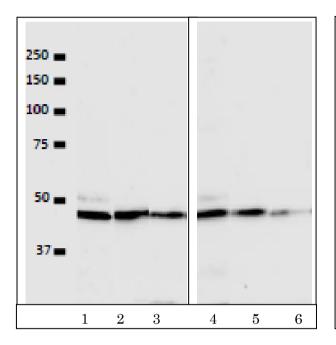
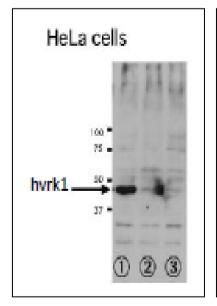


Fig.1. Western blot detection of VRK1 in the crude extracts of human cells. Lanes 1, 2, 3; HeLa cell extract (5x10⁴ cells) with antibody dilutions at 1/100, 1/500, 1/1000. Lanes 4, 5, 6; U2OS cell extract (5x10⁴ cells) with the antibody dilutions at 1/100, 1/500, 1/1,000. As secondary antibody, Alexa488 goat anti-mouse IgG was used. ImmunoStar®LD (Wako, Tokyo) was used as chemiluminescence reagent and images were taken with BIO-RAD ChemiDocXRS.

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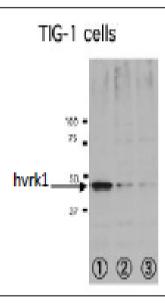


Fig.2. Inhibition of VRK1 expression in human cells treated by RNAi. specific to VRK1.

1; Luciferase RNAi Lane (control). Lane2; VRK1-1 RNAi. 3; VRK1-2 RNAi.. Lane dilution. Antibody at 1/500 Lumi-Light Plus (Roche) was chemiluminescence used reagent. Extracts from 5x104 cells.

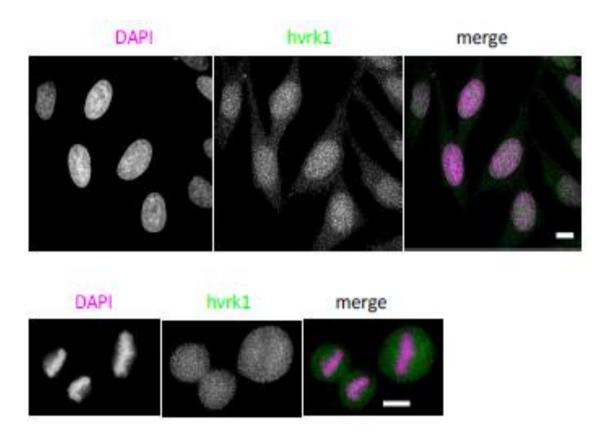


Fig.3. Immunoflorescence staining of VRK1 in HeLa cells, PA fixed.

Top; Interphase cells were fixed with paraformaldehyde and stained with the anti-human VRK1 antibody (hvrk1) at 1/100 dilution (center), DNA was stained with DAPI (left) and two images were merged (right; merge).

Bottom; Metaphase cells. At metaphase, VRK1 dots were solely detected in nuclei.

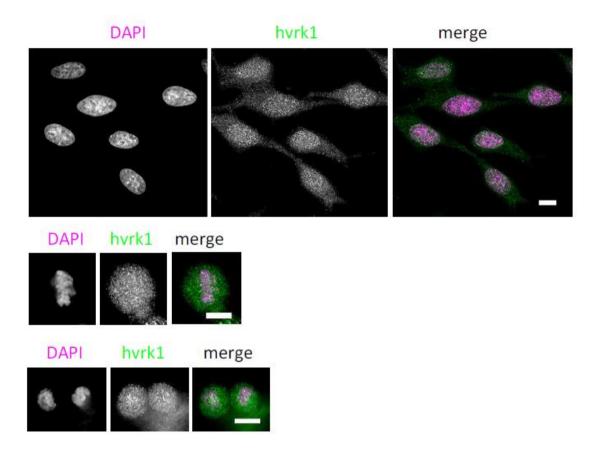


Fig.4. Immunoflorescence staining of VRK1 in HeLa cells, methanol fixed.

Top; Interphase cells were fixed with methanol and stained with the anti-human VRK1 antibody (hvrk1) at 1/100 dilution (center), DNA was stained with DAPI (left) and two images were merged (right; merge).

Center and bottom; Metaphase cells. At metaphase, VRK1 dots were solely detected in nuclei.

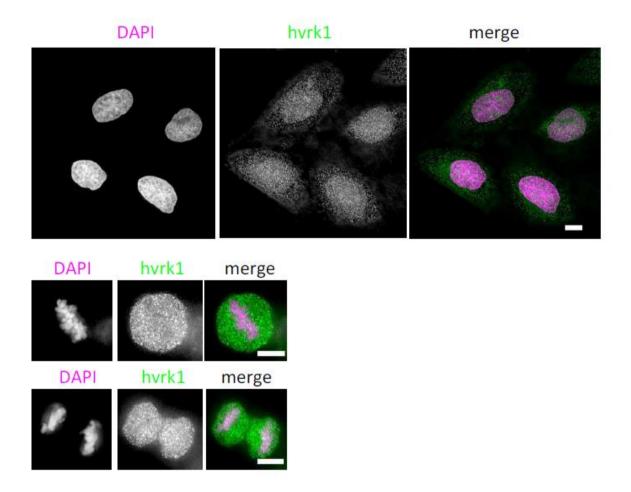


Fig. 5. Immunoflorescence staining of VRK1 in U-2 OS cells, formaldehyde fixed.

Top; Interphase cells were fixed with formaldehyde and stained with the anti-human VRK1 antibody (hvrk1) at 1/100 dilution (center), DNA was stained with DAPI (left) and two images were merged (right; merge).

Center and bottom; Metaphase cells. At metaphase, VRK1 dots were solely detected in nuclei.

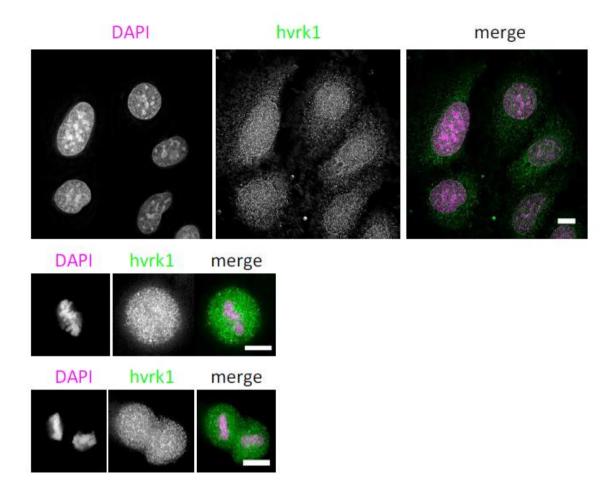


Fig. 6. Immunoflorescence staining of VRK1 in U-2 OS cells, methanol fixed.

Top; Interphase cells were fixed with methanol and stained with the anti-human VRK1 antibody (hvrk1) at 1/100 dilution (center), DNA was stained with DAPI (left) and two images were merged (right; merge).

Center and bottom; Metaphase cells. At metaphase, VRK1 dots were solely detected in nuclei.