PRODUCT INFORMATION

Rabankyrin-5 (human, recombinant)

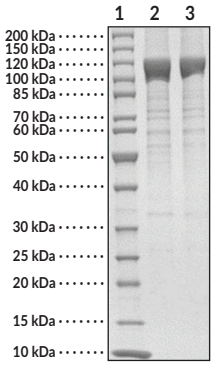
Item No. 45169

Overview and Properties

Synonyms:	Ankyrin Repeat and FYVE Domain-containing Protein 1, Ankyrin Repeats Hooked To A Zinc Finger Motif, Rank-5
Source:	Active recombinant human Rabankyrin-5 expressed in insect cells
Amino Acids:	2-1,169 (full length)
Uniprot No.:	Q9P2R3
Molecular Weight:	128.4 kDa
Storage:	-80°C (as supplied)
Stability:	≥1 year
Purity:	≥90% estimated by SDS-PAGE
Supplied in:	50 mM Tris-HCl, pH 7.5, with 200 mM sodium chloride and 20% glycerol
Endotoxin Testing:	<1.0 EU/µg, determined by the LAL endotoxin assay
Protein Concentration:	***batch specific*** mg/ml
Activity:	***batch specific*** U/ml
Specific Activity:	***batch specific*** U/mg

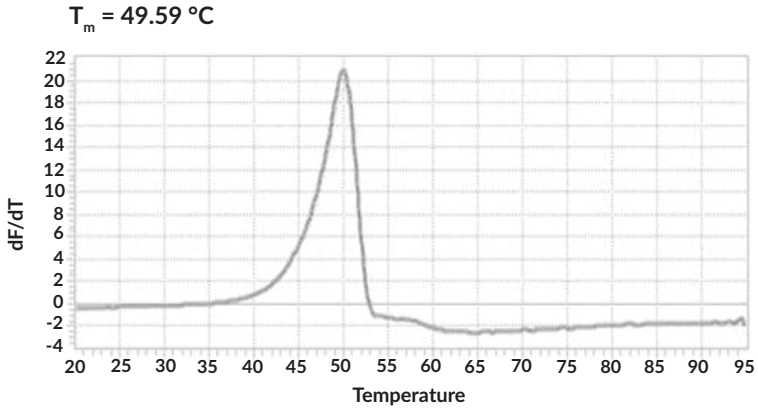
Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Images



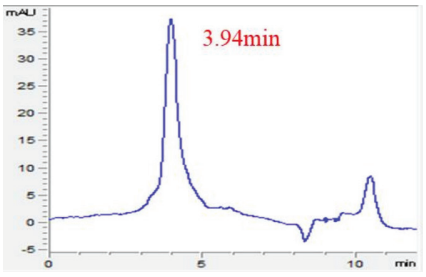
Lane 1: MW Markers
Lane 2: Rabankyrin-5 (2 µg) (reduced)
Lane 3: Rabankyrin-5 (2 µg) (non-reduced)

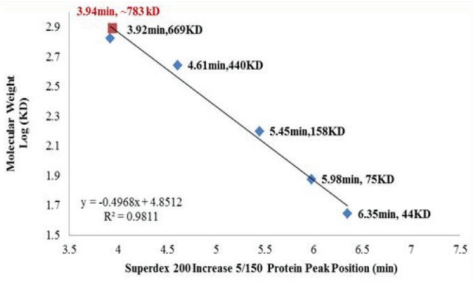
SDS-PAGE Analysis of Rabankyrin-5.



Protein melting thermal profile of Rabankyrin-5.

Analytical SEC





Analytical size exclusion chromatography of purified protein Rabankyrin-5.

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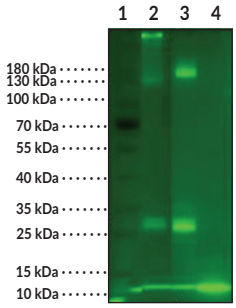

Lane 1: MW Markers
Lane 2: BODIPY-Ub + UBE1 + UbcH5b + Rabankyrin-5 with ATP
Lane 3: BODIPY-Ub + UBE1 + UbcH5b with ATP
Lane 4: BODIPY-Ub + UBE1 + UbcH5b without ATP

Gel based ligase assay. The ubiquitin conjugating activity of Rabankyrin-5 was validated through its ability to catalyze the generation of polyubiquitin chains in the presence of the E1 activating enzyme UBE1, the E2 conjugating enzyme UbcH5b, and BODIPY-ubiquitin. Incubation of Rabankyrin-5 for 60 minutes at 37˚C in the presence of BODIPY-ubiquitin, UBE1, UbcH5b and ATP (Lane 2) was compared alongside two control reactions with either Rabankyrin-5 (Lane 3) or Rabankyrin-5 with ATP (Lane 4) excluded from the reaction. Ubiquitin conjugates were identified by the migration of the Bodipy-ubiquitin band and these were observed only in the presence of ATP and Rabankyrin-5.

Description

Rabankyrin-5 is a Rab5 effector protein with roles in endosomal fusion, micropinocytosis, and mitochondrial homeostasis and dynamics.[1,2] It is composed of an N-terminal BTB domain, 21 ankyrin (ANK) repeats, and a C-terminal FYVE finger domain and primarily localizes to endosomal membranes, but can also localize to the cytosol.[1] Rabankyrin-5 localizes with Rab5-positive early endosomes, and immunodepletion of Rabankyrin-5 using anti-Rabankyrin-5 antibodies inhibits early endosome fusion in HeLa cell cytosol. Knockdown of *ANKFY1*, the gene encoding Rabankyrin-5, using endoribonuclease-prepared small interfering RNA (esiRNA) inhibits EGF-induced micropinocytosis in skin cancer cells. *ANKFY1* knockdown also induces mitochondrial elongation and stasis in retinal pigment epithelial (RPE) cells.[3] Cayman's Rabankyrin-5 (human, recombinant) protein has a calculated molecular weight of 128.4 kDa.

References

1. Schnatwinkel, C., Christoforidis, S., Lindsay, M.R., *et al.* The Rab5 effector Rabankyrin-5 regulates and coordinates different endocytic mechanisms. *PLoS Biol.* **2(9),** E261 (2004).
2. Farmer, T., Naslavsky, N., and Caplan, S. Tying trafficking to fusion and fission at the mighty mitochondria. *Traffic* **19(8),** 569-577 (2018).
3. Farmer, T., Reinecke, J.B., Xie, S., *et al.* Control of mitochondrial homeostasis by endocytic regulatory proteins. *J. Cell. Sci.* **130(14),** 2359-2370 (2017).

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