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Catalog #	Aliquot Size
R297-380G-20	20 µg
R297-380G-50	50 µg

RNF34L (CARP2), Active

Full-length human recombinant protein expressed in *Sf*9 cells

Catalog # R297-380G

Lot # V2538-5

Product Description

Recombinant full-length human RNF34L was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The RNF34L gene accession number is NM_001017368.

Gene Aliases

FRING; CARP2; CARP-2; RNF189; RNF34L; RIFIFYLIN

Formulation

Recombinant proteins stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

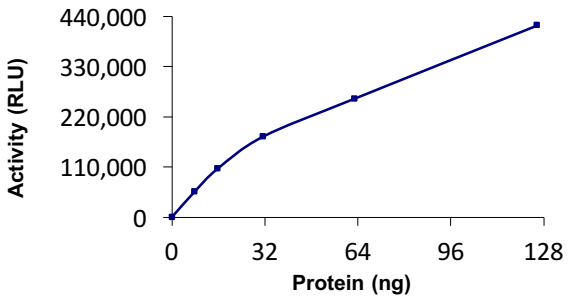
Scientific Background

E3 ubiquitin-protein ligase RNF34L, also named CARP2 (Caspases-8/10 Associated RING protein 2), acts as RING-domain E3 ligases, ubiquitinates apical caspases and targets them for proteasome-mediated degradation. CARP gene silencing inhibits tumor cell survival and increases cancer cell sensitivity to the death ligand or chemotherapy-induced apoptosis. CARP proteins target the tumor suppressor p53 for degradation. RNF34L also acts at the level of endocytic vesicles to limit the intensity of TNF-induced NF-κB activation by the regulated elimination of the adaptor protein RIP1.

References

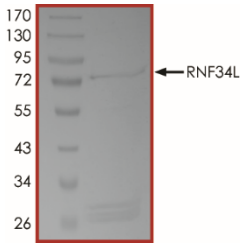
1. Liao, W. et al: CARP-2 is an endosome-associated ubiquitin ligase for RIP and regulates TNF-induced NF-kappaB activation. Curr Biol. 18(9):641-649, 2008
2. McDonald, E.R. 3rd et al: Suppression of caspase-8- and -10-associated RING proteins results in sensitization to death ligands and inhibition of tumor cell growth. PNAS. 20;101(16):6170-6175, 2004
3. Yang, W. et al: CARPs are E3 ligases that target apical caspases and p53. Cancer Biol. Ther. 6(11):1676-1683, 2007

Specific Activity



The specific activity of RNF34L was determined to be **18 nmol /min/mg** as per activity assay protocol.

Purity



The purity of RNF34L was determined to be **>70%** by densitometry, approx. MW **73 kDa**.

RNF34L (CARP2), Active

Full-length recombinant human protein expressed in *Sf*9 cells

Catalog #	R297-380G
Specific Activity	18 nmol/min/mg
Lot #	V2538-5
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes

Active RNF34L (Catalog #: R297-380G), UBA1 (Catalog #: U201-380G) and UBE2D3 (Catalog #: U215-380H) diluted with Ubiquitination Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active RNF34L for optimal results).

Ubiquitination Buffer

Buffer components: 40mM Tris (pH7.5), 20mM $MgCl_2$, 0.1mg/ml BSA. Add 0.5mM DTT prior to use.

AMP-Glo™ Assay (Promega, Catalog #: V5011)

AMP, 10 mM
Ultra Pure ATP, 10mM
AMP-Glo™ Reagent I
AMP-Glo™ Reagent II
Kinase-Glo™ One Solution

Substrate (Catalog #: U06-54N)

Wild-type ubiquitin protein diluted with Ubiquitination Buffer to a working stock of 170ng/µl (20µM).

Assay Protocol

The RNF34L assay is performed using the AMP-Glo™ Assay kit (Promega), by detecting the amount of the universal AMP generated. Ubiquitin conjugation is proportional to the generated AMP, and the presence of all components of the Ub conjugation machinery (Ub, E1, E2, and E3) is required for maximal activity of the system.

Step 1. Thaw the active RNF34L, UBA1, UBE2D3 and ubiquitin on ice, and all AMP-Glo™ components except AMP-Glo™ Reagent II at room temperature. Keep AMP-Glo™ Reagent II on ice.

Step 2. Prepare the following working solutions with Ubiquitination Buffer:

- 2X Reaction Cocktail: 170ng/µl ubiquitin + 15ng/µl UBA1 + 14ng/µl UBE2D3 + 50µM ATP
- 2X final concentration of Active RNF34L

Step 3. In a half-area white 96-well plate, add the following components to bring the initial reaction volume to 10 µl:

Component 1. 5 µl of 2X Reaction Cocktail

Component 2. 5 µl of 2X Active RNF34L

Note: A blank control can be set up as outlined above by replacing the enzyme working solution with an equal volume of Ubiquitination Buffer.

Step 4. Briefly centrifuge the plate to ensure reagents are fully mixed and at the bottom of the wells. Seal the plate with a plate seal and incubate at 37°C for 60 minutes

Step 5. Equilibrate plate to room temperature. Add 10 µl of AMP-Glo™ Reagent I to all wells, mix by shaking for 1-2 minutes. Incubate the plate at room temperature for 60 minutes.

Step 6. Prepare AMP Detection Solution by adding AMP-Glo™ Reagent II to Kinase-Glo™ One Solution at a 1:100 volume ratio. Add 20 µl of the Detection Solution to all wells. Mix for 1-2 minutes and incubate at room temperature for 30 minutes

Step 7. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 8. Using the AMP standard curve, determine the concentration of AMP produced (µM) and calculate the enzyme specific activity as outlined below. For a detailed protocol of how to determine AMP amount from RLUs, see AMP-Glo™ Assay protocol at Promega's website: www.promega.com/protocols

Enzyme Specific Activity (SA) (nmol/min/mg)

$$= \frac{[AMP](\mu M) \times Reaction\ Volume(\mu l)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-3}$$

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