RNF34 (CARP), Active
Full-length human recombinant protein expressed in Sf9 cells

Catalog # R296-380G
Lot # L1908-6

Product Description
Recombinant human RNF34 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The RNF34 gene accession number is NM_025126.

Gene Aliases
RNF34; CARP; CARP1; hRFI; RFI; RIF; RIFF

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 RNF34, also named CARP1 (Caspases-8/10 Associated RING protein 1), 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. RNF34 is also a negative regulator of the NOD1 pathway through direct interaction and ubiquitination of NOD1.

References

Specific Activity
The specific activity of RNF34 was determined to be 21 nmol/min/mg as per activity assay protocol.

Purity
The purity of RNF34 was determined to be >80% by densitometry, approx. MW 68 kDa.

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Catalog # Specific Activity
R296-380G 21 nmol/min/mg

Lot # Purity Concentration Stability Storage & Shipping
L1908-6 >80% 0.1 µg/µl 1yr at –70°C from date of shipment

Product shipped on dry ice.

To place your order, please contact us by phone 1-(604)-232-4600, fax 1-604-232-4601 or by email: orders@signalchem.com
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Activity Assay Protocol

Reaction Components

Active Ubiquitinating Enzymes
Active RNF34 (Catalog #: R296-380G), 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 RNF34 for optimal results).

Ubiquitination Buffer
Buffer components: 40mM Tris (pH7.5), 20mM MgCl₂, 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 RNF34 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 RNF34, 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 RNF34

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 RNF34

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)

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

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