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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
B280-380G-20	20 µg
B280-380G-50	50 µg

BIRC3, Active

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

Catalog # B280-380G

Lot # V2408-17

Product Description

Recombinant full-length human BIRC3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The BIRC3 gene accession number is NM_001165.

Gene Aliases

AIP1; API2; c-IAP2; CIAP2; HAIP1; HIAP1; MALT2; MIHC; RNF49

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.

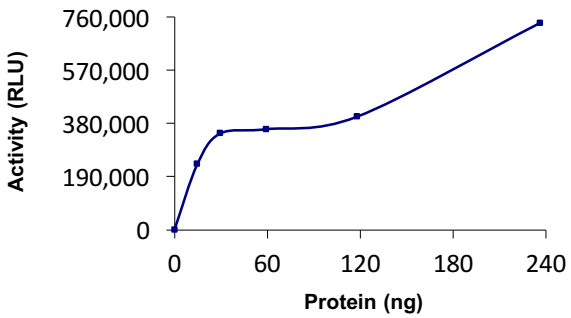
Scientific Background

BIRC3 or baculoviral IAP repeat containing 3 is a member of the IAP family of proteins. BIRC3 inhibit apoptosis by binding to tumor necrosis factor receptor-associated factors TRAF1 and TRAF2 and thereby interfering with activation of ICE-like proteases. BIRC3 inhibits apoptosis induced by serum deprivation but does not affect apoptosis resulting from exposure to menadione, a potent inducer of free radicals. BIRC3 is a potential oncogene which is overexpressed in multiple lung cancers with or without higher copy numbers. BIRC3 is a key regulator of NOD innate immunity signaling.

References

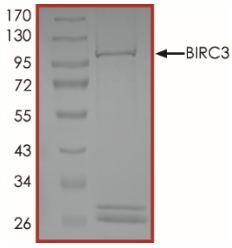
1. Dai, Z. et al: A comprehensive search for DNA amplification in lung cancer identifies inhibitors of apoptosis cIAP1 and cIAP2 as candidate oncogenes. Hum. Mol. Genet. 12: 791-801, 2003
2. Bertrand, M.J. et al: Cellular inhibitors of apoptosis cIAP1 and cIAP2 are required for innate immunity signaling by the pattern recognition receptors NOD1 and NOD2. Immunity 30: 789-801, 2009

Specific Activity



The specific activity of BIRC3 was determined to be **17 nmol /min/mg** as per activity assay protocol.

Purity



The purity of BIRC3 was determined to be **>75%** by densitometry, approx. MW **105 kDa**.

BIRC3, Active

Recombinant full-length human protein expressed in *Sf*9 cells

Catalog #	B280-380G
Specific Activity	17 nmol/min/mg
Lot #	V2408-17
Purity	>75%
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 BIRC3 (Catalog #: B280-380G), 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 BIRC3 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 BIRC3 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 BIRC3, 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 BIRC3

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 BIRC3

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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