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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
B281-380G-20	20 µg
B281-380G-50	50 µg

BIRC7, Active

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

Catalog # B281-380G
Lot # L1931-7

Product Description

Recombinant full-length human BIRC7 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The BIRC7 gene accession number is BC014475.

Gene Aliases

KIAP; LIVIN; ML-IAP; MLIAP; RNF50

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.

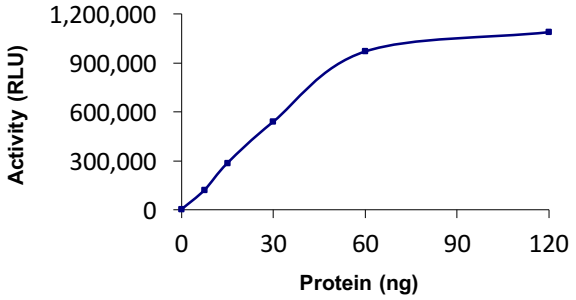
Scientific Background

Baculoviral IAP repeat-containing protein 7 (BIRC7) is a member of the newly discovered inhibitor of apoptosis proteins (IAPs) family whose activity is mediated through the inhibition of CASP3, CASP7 and CASP9, as well as by its E3 ubiquitin-protein ligase. Highly expressed in tumor tissues and inhibits tumor cell apoptosis through blocking the ability of DIABLO/SMAC to disrupt XIAP/BIRC4-caspase interactions. BIRC7 expression in breast cancer correlated with the clinical stage and axillary lymph node metastasis, and promotes breast cancer metastasis through the activation of AKT signaling and induction of EMT in breast cancer cells.

References

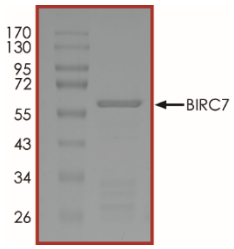
1. Ma, L. et al: Livin promotes Smac/DIABLO degradation by ubiquitin-proteasome pathway. Cell Death Differ. 13:2079-2088, 2006
2. Li, F. et al: Livin promotes progression of breast cancer through induction of epithelial-mesenchymal transition and activation of AKT signaling. Cell Signal. 25(6):1413-1422, 2013

Specific Activity



The specific activity of BIRC7 was determined to be **30 nmol /min/mg** as per activity assay protocol.

Purity



The purity of BIRC7 was determined to be **>95%** by densitometry, approx. MW **60 kDa**.

BIRC7, Active

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

Catalog #	B281-380G
Specific Activity	30 nmol/min/mg
Lot #	L1931-7
Purity	>95%
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 BIRC7 (Catalog #: B281-380G), UBA1 (Catalog #: U201-380G) and UBE2D1 (Catalog #: U213-380H) 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 BIRC7 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 BIRC7 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 BIRC7, UBA1, UBE2D1 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 UBE2D1 + 50µM ATP
- 2X final concentration of Active BIRC7

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 BIRC7

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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