BIRC2 [GST-tagged]

E3 Ligase

Alternate Names: API1, Baculoviral IAP repeat containing protein 2, cIAP1, HIAP2, MIHB

Cat. No.	**63-0015-025**	Quantity:	25 µg
Lot. No.	**30214**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

CERTIFICATE OF ANALYSIS Page 1 of 2

Background

The enzymes of the ubiquitylation pathway play a pivotal role in a number of cellular processes including the regulated and targeted proteasome-dependent degradation of substrate proteins. Three classes of enzymes are involved in the process of ubiquitylation; activating enzymes (E1s), conjugating enzymes (E2s) and protein ligases (E3s). Baculoviral IAP repeat containing protein 2 (BIRC2) is a member of the E3 protein ligase family and cloning of the human gene was first described by Rothe *et al.* (1995). BIRC2 is a RING finger domain ubiquitin E3 ligase that has been shown to ubiquitylate TRAF2 in TNF stimulated Jurkat cell lines (Li *et al.,* 2002). Increased expression of HTRA2 induced by p53 results in the cleavage of BIRC2 and activation of apoptosis (Jin *et al.,* 2003). BIRC2 has been shown to form part of a cytokine receptor signalling complex which also includes, Tnf Receptor-Associated Protein 2 (TRAF2), TRAF3, Ube2N, BIRC1, Inhibitor of Kappa Kappa gamma (IKKγ) and Mitogen Activated Protein Kinase Kinase Kinase 1 (MAP3K1). Activation of the kinases in this complex and translocation of the complex from the membrane to the cytosol was dependent upon TRAF3 degradation by BIRC1/BIRC2 (Matsuzawa *et al.,* 2008). A20 can inhibit BIRC2, TRAF6 and TRAF2 E3 ligase activity in the NFkbeta inflammatory signalling pathway by preventing its interaction with Ube2N and Ube2D3. (Shembade *et al.,* 2010). In macrophages from BIRC2 null mice cytokine and chemokine production is reduced. Activation of NFkbeta and

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Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 25 µg

Concentration: 0.5 mg/ml

Formulation: 50 mM HEPES pH 7.5,
150 mM sodium chloride,
2 mM dithiothreitol, 10% glycerol

Molecular Weight: ~97 kDa

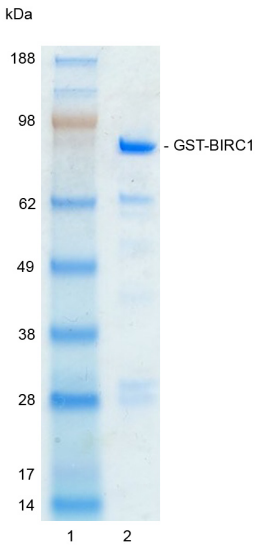
Purity: >62% by InstantBlue™ SDS-PAGE

Stability/Storage: 12 months at -70˚C;
aliquot as required

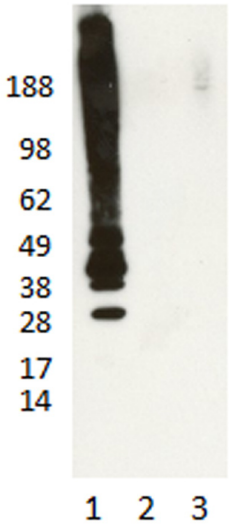
Protein Sequence: Please see page 2

Quality Assurance

Purity:
4-12% gradient SDS-PAGE
InstantBlue™ staining
Lane 1: MW markers
Lane 2: 1 µg GST-BIRC1



Protein Identification:
Confirmed by mass spectrometry.



E3 ligase assay: The ubiquitin conjugating activity of GST-BIRC2 was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme 6His-UBE1, the E2 conjugating enzyme His-UBE2D3 (UbcH5c) (several E2s were tested, data generated with this E2 is provided by way of example) and ubiquitin. Incubation of GST-BIRC2 for 30 minutes at 30˚C in the presence of ubiquitin, 6His-Ube1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-BIRC2 (Lane 3) excluded from the reaction. Ubiquitin conjugates were identified by Western blotting using an anti-ubiquitin conjugate antibody and these were observed only in the presence of both ATP and GST-BIRC2.

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0020
US Toll-Free: 1-888-4E1E2E3 (1-888-431-3233)
Email: sales.support@ubiquigent.com

UK HQ and TECHNICAL SUPPORT
International: +44 (0) 1382 381147 (9AM-5PM UTC)
US/Canada: +1-617-245-0020 (9AM-5PM UTC)
Email: tech.support@ubiquigent.com

Email **services@ubiquigent.com** for enquiries regarding compound profiling and/or custom assay development services.

Lot-specific COA version tracker: v1.0.0

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Background

Continued from page 1

Mapk induced by ubiquitylated RipK2 is reduced due to the lack of BIRC2. BIRC2 null mice also showed resistance to peritonitis induction and it is thought that BIRC2 is a key regulator of the Nucleotide-binding Oligomerization Domain receptor (NOD) innate immune response (Bertrand *et al.,* 2009). More recently BIRC2 has been identified as an onocogene and expression has been found to be increased in squamous cell carcinoma of the cervix (Choschzick *et al.,* 2012).

References:

Bertrand MJM, Doiron K, Labbe K, Korneluk R G, Barker P A, Saleh M (2009) 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.

Choschzick M, Tabibzada AM, Gieseking F, Woelber L, Jaenicke F, Sauter G, Simon R (2012) BIRC2 amplification in squamous cell carcinomas of the uterine cervix. *Virchows Arch* **461**, 123-8.

Jin S, Kalkum M, Overholtzer M, Stoffel A, Chait BT, Levine AJ (2003) CIAP1 and the serine protease HTRA2 are involved in a novel p53-dependent apoptosis pathway in mammals. *Genes Dev* **17**, 359-367.

Li X, YangY, Ashwell JD (2002) TNF-RII and c-IAP1 mediate ubiquitination and degradation of TRAF2. *Nature* **416**, 345-349.

Matsuzawa A, Tseng PH, Vallabhapurapu S, Luo JL, Zhang W, Wang H, Vignali D AA, Gallagher E, Karin M (2008) Essential cytoplasmic translocation of a cytokine receptor-assembled signaling complex. *Science* **321**, 663-668.

Rothe M, Pan MG, Henzel WJ, Ayres TM, Goeddel DV (1995) The TNFR2 TRAF signaling complex contains two novel proteins related to baculoviral inhibitor of apoptosis proteins. *Cell* **83**, 1243-1252.

Shembade N, Ma A, Harhaj E W (2010) Inhibition of NF-kappa-B signaling by A20 through disruption of ubiquitin enzyme complexes. *Science* **327**, 1135-1139.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCHK
TYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGSPGIPGSTRAAA***M***HKTASQRLFPGPSY
QNIKSIMEDSTILSDWTNSNKQKMKYDFS
CELYRMSTYSTFPAGVPVSERSLARAGFYYT
GVNDKVKCFCCGLMLDNWKLGDSPIQKHKQ
LYPSCSFIQNLVSASLGSTSKNTSPMRNS
FAHSLSPTLEHSSLFSGSYSSLSPNPLN
SRAVEDISSSRTNPYSYAMSTEEARFLTY
HMWPLTFLSPSELARAGFYYIGPGDRVAC
FACGGKLSNWEPKDDAMSEHRRHFPNCP
FLENSLETLRFSISNLSMQTHAARMRTFMY
WPSSVPVQPEQLASAGFYYVGRNDDVKCFC
CDGGLRCWESGDDPWVEHAKWFPRCEF
LIRMKGQEFVDEIQGRYPHLLEQLLSTSDT
TGEENADPPIIHFGPGESSSEDAVMMNTPV
VKSALEMGFNRDLVKQTVQSKILTTGENYKT
VNDIVSALLNAEDEKREEEKEKQAEEMASD
DLSLIRKNRMALFQQLTCVLPILDNLLKAN
VINKQEHDIIKQKTQIPLQARELIDTILVK
GNAAANIFKNCLKEIDSTLYKNLFVDKNM
KYIPTEDVSGLSLEEQLRRLQEERTCKVCM
DKEVSVVFIPCGHLVVCQECAPSLRKCPI
CRGIIKGTVRTFLS

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
BIRC2 (regular text): Start ***bold italics*** (amino acid residues 1-618)
Accession number: NP_001157

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