

BIRC2 [GST-tagged]

E3 Ligase

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

Cat. No. 63-0015-025

Lot. No. 30214

Quantity: 25 µg

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 (IKK γ) and Mitogen Activated Protein 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 NF κ B 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 NF κ B and

Continued on page 2

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

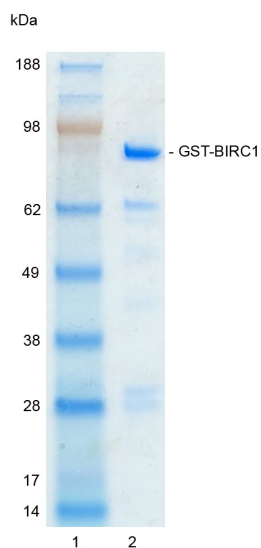
Purity: >62% by InstantBlue™ SDS-PAGE

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

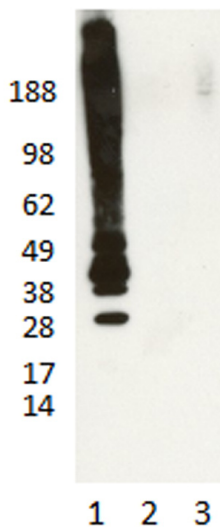
Protein Sequence: Please see page 2

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.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0

BIRC2 [GST-tagged]

E3 Ligase

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

Cat. No. 63-0015-025

Lot. No. 30214

Quantity: 25 µg

Storage: -70°C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS



CERTIFICATE OF ANALYSIS Page 2 of 2

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 oncogene and expression has been found to be increased in squamous cell carcinomas 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, Tabibzade AM, Gieseck 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, Yang Y, 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
CLDAFPKLVCFKKRIEAIPOIDKYLKSSKY
IAWPLQGWQATFGGGDHPKSDLEVLVQ
PLGSPGIPGSTRAAAMHKTASQRLFPGPSY
QNIKSI MEDSTILSDWTNSNKQKMKYDFS
CELYRMSTYSTFPAGVPVRSERLARAGFYT
GVNDKVKCFCCGLMLDNWKLGDSPIQKHQ
LYPSCSFIQNLVSASL GSTSKNTSPMRNS
FAHLSPTLEHSSLSFGSYSSLSPNPLN
SRAVEDISSRTPNYSYAMSTEEARFLTY
HMWPLTFLSPSELARAGFYIIGPGDRVAC
FACGGKLSNWEPKDDAMSEHRRHPNCP
FLENSLETLRFSISNLSMQTHAARMRTFMY
WPSSVPVQPEQLASAGFYVGRNDDVKCFC
CDGGLRCWESGDDPWVEHAKWFP RCEF
LIRMKGQEFVDEIQGRYPHLLLEQLLSTSDT
TGEENADPPIIHFPGGESSEDAVMNTPV
VKSALEMGFNRDLVKQTVQSKILTGTENYKT
VNDIVSALLNAEDEKREEEKEKQAEEMASD
DLSLIRKNRMALFQQLTCVLPILDNLLKAN
VINKQEHDIKQKTQIPLQARELIDTILVK
GNAANIFKNCLKEIDSTLYKNLFVDKNM
KYIPTEDVSGLSLEEQLRRLQEBERTCKVCM
DKEVSVVFI PCGHLVVCQECAPSLRKCPI
CRGIKGTVRTFLS

Tag (**bold text**): N-terminal GST

Protease cleavage site: PreScission™ (LEVLQ▼GP)

BIRC2 (regular text): Start **bold italics** (amino acid residues 1-618)

Accession number: NP_001157



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.

© Ubiquigent 2014. Unless otherwise noted, Ubiquigent, Ubiquigent logo and all other trademarks are the property of Ubiquigent, Ltd.

Limited Terms of Use: For research use only. Not for use in humans or for diagnostics. Not for distribution or resale in any form, modification or derivative OR for use in providing services to a third party (e.g. screening or profiling) without the written permission of Ubiquigent, Ltd.

Lot-specific COA version tracker: v1.0.0