A20 CD(1-366) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: TNFAIP3, OTUD7C, Zinc finger protein A20

Cat. No.	**64-0047-050**	Quantity:	50 µg
Lot. No.	**30163**	Storage:	-70˚C

FOR RESEARCH USE ONLY | NOT FOR USE IN HUMANS | **CERTIFICATE OF ANALYSIS Page 1 of 2**

Background

Deconjugating enzymes (DCEs) are proteases that process ubiquitin or ubiquitin-like gene products, reverse the modification of proteins by a single ubiquitin or ubiquitin-like protein (UBL) and remodel polyubiquitin (or poly-UBL) chains on target proteins (Reyes-Turcu *et al.,* 2009). The deubiquitylating – or deubiquitinating – enzymes (DUBs) represent the largest family of DCEs and regulate ubiquitin dependent signalling pathways. The activities of the DUBs include the generation of free ubiquitin from precursor molecules, the recycling of ubiquitin following substrate degradation to maintain cellular ubiquitin homeostasis and the removal of ubiquitin or ubiquitin-like proteins (UBL) modifications through chain editing to rescue proteins from proteasomal degradation or to influence cell signalling events (Komander *et al.,* 2009). There are two main classes of DUB, cysteine proteases and metalloproteases. A20 is a cysteine protease and is a member of the OTU (ovarian tumour) superfamily of proteins (Balakirev *et al.,* 2003). Cloning of the human gene was first described by Opipari *et al.* (1990). A20, or TNFAIP3, is a ubiquitin-editing enzyme that contains both ubiquitin ligase and deubiquitylase activities. The N-terminal domain of A20 functions as a deubiquitylating enzyme by removing K63-linked ubiquitin chains from receptor-interacting protein (RIP) essential mediator of the proximal TNF receptor-1 (TNFR1) signalling complex. The C-terminal domain of A20, composed of 7 C2/C2 zinc fingers, then functions as a ubiquitin ligase by polyubiquitylating RIP with K48-linked ubiquitin chains, thereby targeting RIP for proteasomal degradation (Wertz *et al.,* 2004). Literature evidence suggests a mechanism of action for A20 in the inhibition of inflammatory signalling pathways.

Continued on page 2

Physical Characteristics

Species: human

Source: *E. coli*

Quantity: 50 µg

Concentration: 0.5 mg/ml

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

Molecular Weight: ~70.9 kDa

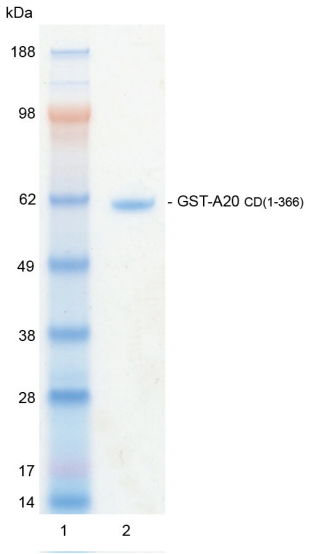
Purity: >98% by InstantBlue™ SDS-PAGE

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-A20 CD(1-366)



Protein Identification:
Confirmed by mass spectrometry.

Deubiquitylase Enzyme Assay:
The activity of GST-A20 CD(1-366) was validated by the monitoring of mono-ubiquitin generation as a result of the enzyme catalysed cleavage of K48-linked di-ubiquitin. Incubation of the substrate in the presence or absence of GST-A20 CD(1-366) was compared confirming the deubiquitylating activity of GST-A20 CD(1-366).

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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

A20 CD(1-366) [GST-tagged]

Deconjugating enzyme: Deubiquitylase

Alternate Names: TNFAIP3, OTUD7C, Zinc finger protein A20

Cat. No.	**64-0047-050**	Quantity:	50 µg
Lot. No.	**30163**	Storage:	-70˚C

FOR RESEARCH USE ONLY NOT FOR USE IN HUMANS

Background

Continued from page 1

Research showed that A20 inhibits the E3 ligase activities of TRAF6, TRAF2, and cIAP1 by antagonizing interactions with E2 ubiquitin-conjugating enzymes Ube2N and Ube2D3. A20, together with the regulatory molecule TAX1BP1, interacted with Ube2N and Ube2D3 and triggered their ubiquitylation and proteasome-dependent degradation (Shembade *et al.,* 2010). More recently, studies have highlighted the clinical and biological importance of A20. Human genetic research has shown strongly linked polymorphisms and mutations in the gene encoding A20 to inflammatory, autoimmune and malignant diseases. Furthermore, studies in gene-targeted mice have revealed that A20 regulates multiple immune cell functions and prevents experimental diseases that closely mimic human conditions. These studies reveal the importance of A20-mediated regulation of ubiquitin-dependent signalling in human disease (Ma and Malynn, 2012).

References:

Balakirev MY, Tcherniuk SO, Jaquinod M and Chroboczek J (2003) Otubains: a new family of cysteine proteases in the ubiquitin pathway. *EMBO Rep* **4**, 517-522.

Komander D, Clague MJ and Urbe S (2009) Breaking the chains: structure and function of the deubiquitinases. *Nat Rev Mol Cell Biol* **10**, 550-563.

Ma A and Malynn BA (2012) A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nature Reviews. Immunology* **12**, 774-785.

Opipari AW, Jr., Boguski MS and Dixit VM (1990) The A20 cDNA induced by tumor necrosis factor alpha encodes a novel type of zinc finger protein. *J Biol Chem* **265**, 14705-14708.

Reyes-Turcu FE, Ventii KH and Wilkinson KD (2009) Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. *Ann Rev Biochem* **78**, 363-397.

Shembade N, Ma A and Harhaj EW (2010) Inhibition of NF-kappaB signaling by A20 through disruption of ubiquitin enzyme complexes. *Science* **327**, 1135-1139.

Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, et al. (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* **430**, 694-699.

Physical Characteristics

Continued from page 1

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKYEEH
LYERDEGDKWRNKKFELGLEFPNLPYYIDGD
VKLTQSMAIIRYIADKHNMLGGCPKERAEISM
LEGAVLDIRYGVSRIAYSKDFETLKVDFL
SKLPEMLKMFEDRLCHKTYLNGDHVTHPD
FMLYDALDVVLYMDPMCLDAFPKLVCFK
KRIEAIPQIDKYLKSSKYIAWPLQGWQATFG
GGDHPPKSDLEVLFQGPLGSPGIPGSTRAAA
MAEQVLPQALYLSNMRKAVKIRERTPED
IFKPTNGIIHHFKTMHRYTLEMFRTC
QFCPQFREIIHKALIDRNIQATLESQKKL
NWCREVRKLVALKTNGDGNCLMHATSQYM
WGVQDTDLVLRKALFSTLKETDTRNFK
FRWQLESLKSQEFVETGLCYDTRNWNDEWDN
LIKMASTDTPMARSGLQYNSLEEIHIFVL
CNILRRPIIVISDKMLRSLESGSNFAPLKVG
GIYLPLHWPAQECYRYPIVLGYDSHHFV
PLVTLKDSGPEIRAVPLVNRDRGRFEDLKVH
FLTDPENEMKEKLLKEYLMVIEIPVQGWD
HGTTHLINAAKLDEANLPKEINLVDDY
FELVQHEYKKWQENSEQGRRE

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
A20 CD(1-366) (regular text): Start ***bold italics*** (amino acid residues 1-366)
Accession number: NP_006281

www.ubiquigent.com
Dundee, Scotland, UK

ORDERS / SALES SUPPORT
International: +1-617-245-0003
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