ITCH [GST-tagged]

E3 Ligase

Alternate Names: AIF4, AIP4, Atrophin1 interacting protein 4, dJ468O1.1, EC 6.3.2, NAPP1, NFE2 associated polypeptide 1, Ubiquitin protein ligase ITCH

Cat. No.	**63-0006-025**	Quantity:	25 µg
Lot. No.	**1423**	Storage:	-70˚C

FOR RESEARCH USE ONLY

NOT FOR USE IN HUMANS

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). Itchy E3 ubiquitin protein ligase (ITCH) is a member of the E3 protein ligase family and cloning of the human gene was first described by Wood *et al.* (1998). ITCH belongs to the HECT family of E3 ubiquitin-ligases that regulate key trafficking decisions, including targeting of proteins to the proteasome or lysosomes. ITCH protein contains an N-terminal C2 domain, four tandem WW domains and a HECT (homologous to the E6 associated protein carboxyl terminus) domain. ITCH has been shown to mediate the ubiquitylation of EGF and CXCR4 receptors, targeting them for degradation (Azakir and Angers 2009; Marchese *et al.*, 2003). JNK phosphorylates ITCH activating its E3 ligase activity, which in turn controls the turnover of Jun proteins and T cell differentiation. The activity of ITCH ubiquitin ligase is negatively regulated by Fyn catalysed tyrosine phosphorylation of ITCH Tyr371 (Yang *et al.* 2006).

References:

Azakir BA, Angers A (2009) Reciprocal regulation of the ubiquitin ligase Itch and the epidermal growth factor receptor signaling. *Cell Signal* **21**, 1326-36.

Marchese A, Raiborg C, Santini F, Keen JH, Stenmark H, Benovic JL (2003) The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4. *Dev Cell* **5**, 709-22.

Wood JD, Yuan J, *et al.* (1998) Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. *Mol Cell Neurosci* **11**, 149-60.

Yang C, Zhou W, Jeon MS, Demydenko D, Harada Y, Zhou H, Liu YC (2006) Negative regulation of the E3 ubiquitin ligase itch via Fyn-mediated tyrosine phosphorylation. *Mol Cell* **21**, 135-41.

Physical Characteristics

Species: human

Source: *E. coli* expression

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: ~125.5 kDa

Purity: >95% by InstantBlue™ SDS-PAGE

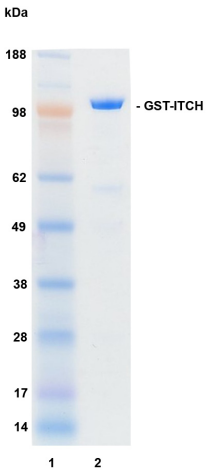
Stability/Storage: 12 months at -70˚C; aliquot as required

Protein Sequence: Please see page 2

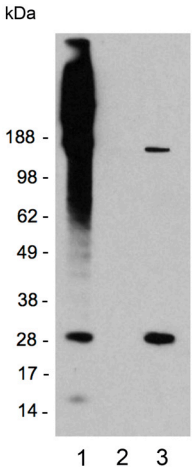
Quality Assurance

Protein Identification:
Confirmed by mass spectrometry.

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



E3 ligase assay:
The ubiquitin conjugating activity of GST-ITCH was validated through its ability to catalyse the generation of polyubiquitin chains in the presence of the E1 activating enzyme His-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-ITCH for 60 minutes at 37˚C in the presence of ubiquitin, His-UBE1, His-UBE2D3 and ATP (Lane 1) was compared alongside two control reactions with either ATP (Lane 2) or GST-ITCH (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-ITCH (with the exception of one species of approximately 140 MW observable in lane 3).



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

Lot-specific COA version tracker: v1.0.1

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Physical Characteristics

Protein Sequence:

MSPILGYWKIKGLVQPTRLLLEYLEEKY
EEHLYERDEGDKWRNKKFELGLEFPN
LPYYIDGDVKLTQSMAIIRYIADKHNMLG
GCPKERAEISMLEGAVLDIRYGVSRIAY
SKDFETLKVDFLSKLPEMLKMFEDRLCH
KTYLNGDHVTHPDFMLYDALDVVLYMDPM
CLDAFPKLVCFKKRIEAIPQIDKYLKSSKY
IAWPLQGWQATFGGGDHPPKSDLEVLFQG
PLGS***M***SDSGSQLGSMGSLTMKSQLQITVI
SAKLKENKKNWFGPSPYVEVTVDGQSK
KTEKCNNTNSPKWKQPLTVIVTPVSKLH
FRVWSHQTLKSDVLLGTAALDIYETLKSN
NMKLEEVVVTLQLGGDKEPTETIGDLSI
CLDGLQLESEVVTNGETTCSESASQND
DGSRSKDETRVSTNGSDDPEDAGAGEN
RRVSGNNSPSLSNGGFKPSRPPRPSRP
PPPTPRRPASVNGSPSATSESDGSSTG
SLPPTNTNTNTSEGATSGLIIPLTISGGS
GPRPLNPVTQAPLPPGWEQRVDQH
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RVDNMGRIYYVDHFTRTTTWQRPTLES
VRNYEQWQLQRSQLQGAMQQFNQRFIYGN
QDLFATSQSKEFDPLGPLPPGWEKRTDSN
GRVYFVNHNTRITQWEDPRSQGQLNEK
PLPEGWEMRFTVDGIPYFVDHNRRTTTY
IDPRTGKSALDNGPQIAYVRDFKAKVQY
FRFWCQQLAMPQHIKITVTRKTLFEDS
FQQIMSFSPQDLRRRLWVIFPGEEGLDYG
GVAREWFFLLSHEVLNPMYCLFEYAG
KDNYCLQINPASYINPDHLKYFRFIGR
FIAMALFHGKFIDTGFSLPFYKRILNK
PVGLKDLESIDPEFYNSLIWVKEN
NIEECDLEMYFSVDKEILGEIKSHDLKPNG
GNILVTEENKEEYIRMVAEWRLSRGVE
EQTQAFFEGFNEILPQQYLQYFDAKELEV
LLCGMQEIDLNDWQRHAIYRHYARTSKQ
IMWFWQFVKEIDNEKRMRLLQFVTGTCR
LPVGGFADLMGSNGPQKFCIEKVGKENWL
PRSHTCFNRLDLPPYKSYEQLKEKLLFA
IEETEGFGQE

Tag (**bold text**): N-terminal GST
Protease cleavage site: PreScission™ (LEVLFQ▼GP)
ITCH (regular text): Start ***bold italics*** (amino acid residues 1-862)
Accession number: NP_1136713

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Lot-specific COA version tracker: v1.0.1