

Certificate of Analysis

SCF^{βTrCP1} complex, active
 (Recombinant E3 ligase expressed in Sf21 insect cells)
 Item # 23-026, 23-026M, 23-026-K
 Parent Lot # D11CP014N

The data presented in this document apply to the parent lot shown above and to all pack sizes derived from subsequent vialling runs of this parent lot. An alphabetical suffix after the parent lot number is used to denote each vialling run.

Product Description: Complex of *N*-terminal GST-tagged, recombinant human βTrCP1 amino acids 53-end, *N*-terminal GST-tagged, recombinant human Skp1 full length, *N*-terminal 6His-tagged, recombinant human Cul1 full length and untagged recombinant human Rbx1 full length, co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Purity 92% by SDS-PAGE and Coomassie blue staining. βTrCP1 MW = 91kDa, Skp1 MW=46kDa, Cul1 MW = 93kDa, Rbx1 MW = 12kDa

Activity (Parent lot# D11CP014N): This lot of SCF^{βTrCP1} complex is active and meets product specifications.

Formulation: 1.504mg/ml of enzyme in 50mM Tris/HCl pH7.5, 300mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

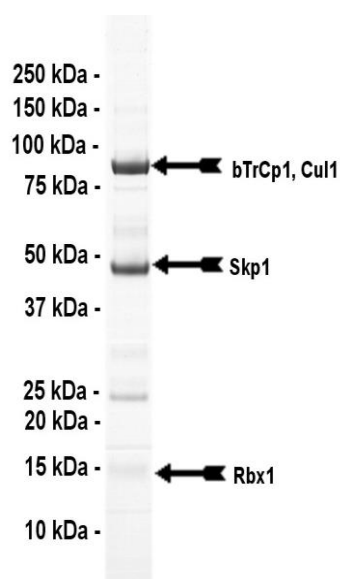
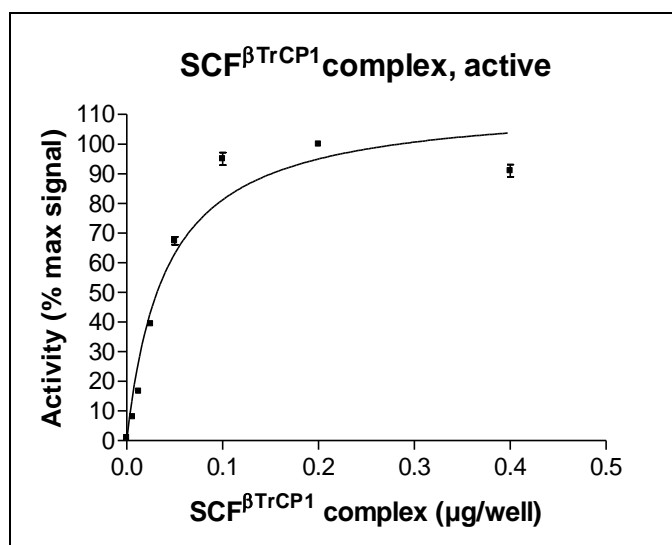
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

Quality Control Testing

Assay: This enzyme was titrated in a ubiquitination assay and the results normalised against the maximum signal.

Protein Identity: Confirmed identity as βTrCP1, Skp1, Cul1 and Rbx1 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3μg of βTrCP1 complex, active.

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E3 Assay Protocol

Reagents:

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|--|---------------------------|
| 1. UBE1, active (Item # 23-021) | 5. 1x Reaction Buffer |
| 2. UbchH3, active (Item # 23-022) | 6. Biotinylated-Ubiquitin |
| 3. SCF ^{βTrCP1} complex, active (Item # 23-026) | 7. Stop Solution |
| 4. IκBα, activated (Item # 23-028) | |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl₂).

SCF^{βTrCP1} complex, active is incubated with 25mM MOPS pH 7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, 10nM UBE1, 500nM UbchH3, 25nM IκBα and 2μM biotinylated-ubiquitin. The reaction is initiated with the addition of biotinylated-ubiquitin. After 30 minutes at room temperature the reaction is terminated by the addition of 25mM MOPS pH 7.5 containing 125mM EDTA, 150mM NaCl, and 0.05% Tween 20. Reaction products are separated by capture onto a microplate coated with anti-c-Myc antibody and washing with PBS containing 0.05% Tween 20. SCF^{βTrCP1} complex activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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SCF^{βTrCP1} complex Information

<u>Protein</u>	Complex of human βTrCP1, human Skp1, human Cul1, human Rbx1
<u>Accession number</u>	GenBank NM_033637 βTrCP1, GenBank NM_170679 Skp1, GenBank NM_003592 Cul1, GenBank NM_014248 Rbx1 The recombinant βTrCP1 protein contains the amino acid substitution E353D with reference to GenBank NM_033637.
<u>Alternative Names</u>	SCF-β-TrCP, SCF ^{FWD1}
<u>Key Facts</u>	The SCF (Skp1-Cul1-F-box protein) complexes represent the largest family of ubiquitin-protein ligases and mediate the ubiquitination of a broad spectrum of regulatory and signalling proteins in diverse cellular pathways. The SCF consists of three invariant components, Skp1, Cul1, and Rbx1 and an interchangeable subunit, an F-box protein which is responsible for recruiting specific substrates to be ubiquitinated by the SCF. SCF ^{βTrCP1} mediates the ubiquitination and subsequent proteasomal degradation of a variety of proteins involved in various cellular processes, these include IκBα, an inhibitor of the transcription factor NFκB and β-catenin which is involved in the Wnt signalling pathway. A number of cancers, including those in breast, colon and pancreatic tissue have been associated with the deregulation of SCF ^{βTrCP1} .
<u>Related Products</u>	Item # 23-021 UBE1, active, Item # 23-022 UbcH3, active, Item # 23-025 UbcH4, active, Item # 23-027 β-catenin, activated, Item # 23-028 IκBα, activated
<u>Selected References</u>	<p>Willems A. R. <i>et al.</i> A Hitchhiker's Guide to the Cullin Ubiquitin Ligases: SCF and its Kin. <i>Biochimica et Biophysica Acta.</i>, 1695: 133-170, 2004</p> <p>Frescas D. and Pagano M. Deregulated Proteolysis by the F-box Proteins SKP2 and β-TrCP: tipping the scales of cancer. <i>Nat Rev Cancer</i>, 8: 438–449, 2008</p> <p>Ougolkov A. <i>et al.</i>, Associations Among β-TrCP, an E3 Ubiquitin Ligase Receptor, β-catenin, and NFκB in Colorectal Cancer. <i>J Natl Cancer Inst.</i> 96: 1161-1170, 2004</p> <p>Kitagawa M. <i>et al.</i>, An F-box protein, FWD1, Mediates Ubiquitin Dependent Proteolysis of β-catenin. <i>The EMBO Journal</i>, 18: 2401-2410, 1999</p> <p>Strack P. <i>et al.</i>, SCF^{βTrCP} and Phosphorylation Dependent Ubiquitination of IκBα Catalysed by Ubc3 and Ubc4. <i>Oncogene</i>, 19: 3529-3536, 2000</p>

Reviewed and approved by site quality representative.

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