

Certificate of Analysis

SCF^{Fbw7} complex, active
(Recombinant E3 ligase expressed in Sf21 insect cells)
Item # 23-030, 23-030-K, 23-030M
Parent Lot # D11BP021N

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 Fbw7 full length, *N*-terminal 6His-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 93% by SDS-PAGE and Coomassie blue staining. Fbw7 MW = 107kDa, Skp1 MW = 21kDa, Cul1 MW = 93kDa, Rbx1 MW = 12kDa

Activity (Parent lot# D11BP021N): This lot of SCF^{Fbw7} complex is active and meets product specifications.

Formulation: 0.412mg/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.

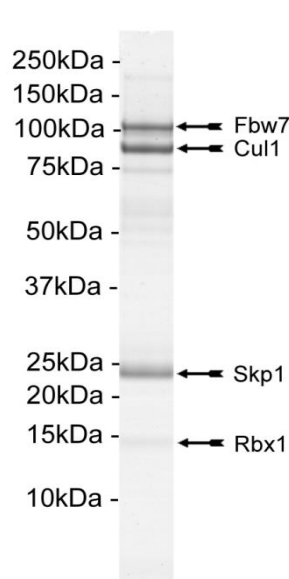
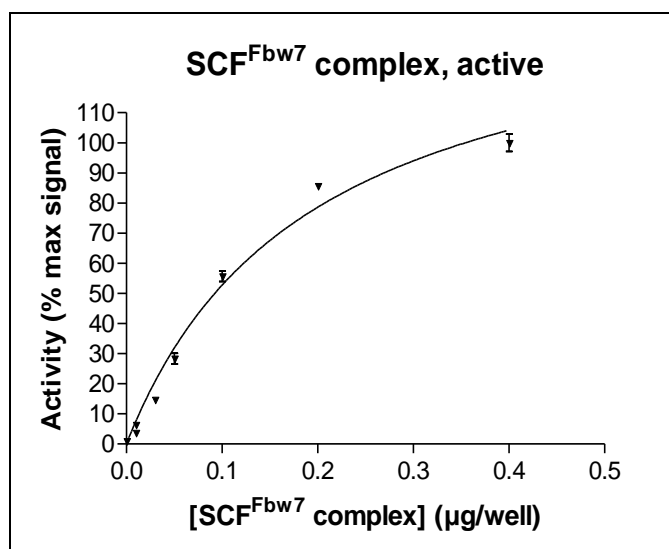
Handling Recommendations: Rapidly thaw the vial under cold water and immediately place on ice. Aliquot unused material into pre-chilled micro-centrifuge tubes and immediately snap-freeze the vials in liquid nitrogen prior to re-storage at -70°C.

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 Fbw7, 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 SCF^{Fbw7} complex, active.

Certificate of Analysis

E3 Assay Protocol

Reagents:

- | | |
|--|---------------------------|
| 1. UBE1, active (Item # 23-021) | 5. 1x Reaction Buffer |
| 2. UbchH3, active (Item # 23-022) | 6. Biotinylated-Ubiquitin |
| 3. SCF ^{Fbw7} complex, active (Item # 23-030) | 7. Stop Solution |
| 4. Cyclin E1 complex (Item # 23-031) | |

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^{Fbw7} complex, active is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10μM ATP, 10nM UBE1, 500nM UbchH3, 100nM Cyclin E1 complex, 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 pH7.5 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^{Fbw7} complex activity is measured by detection of bound ubiquitin via electrochemiluminescence.

Certificate of Analysis

SCF^{Fbw7} complex Information

<u>Protein</u>	Complex of human Fbw7, human Skp1, human Cul1, human Rbx1
<u>Accession number</u>	GenBank NM_033632 Fbw7, GenBank NM_170679 Skp1, GenBank NM_003592 Cul1, GenBank NM_014248 Rbx1
<u>Alternative Names</u>	SCF ^{Fbxw7} , SCF ^{hCdc4}

<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 ^{Fbw7} mediates the ubiquitination and subsequent proteasomal degradation of target proteins involved in cell division, growth and differentiation, including Cyclin E1, c-Myc, Notch and c-Jun. Mutations have been found in the Fbw7 subunit of SCF ^{Fbw7} in various types of human cancers, therefore Fbw7 is considered to be a potent tumour suppressor.
-------------------------	---

<u>Related Products</u>	Item # 23-021 UBE1, active, Item # 23-022 UbchH3, active, Item # 23-029 UbchH5a, active, Item # 23-031 Cyclin E1 complex
--------------------------------	--

Selected References

Willems A. R. *et al.* A Hitchhiker's Guide to the Cullin Ubiquitin Ligases: SCF and its Kin. *Biochimica et Biophysica Acta.*, 1695: 133-170, 2004

Minella A.C. and Clurman B.E. Mechanisms of Tumor Suppression by the SCF^{Fbw7}. *Cell Cycle* 4: 1356-1359, 2005

Welcker M. *et al.*, The Fbw7 Tumor Suppressor Regulates Glycogen Synthase Kinase 3 Phosphorylation-Dependent c-Myc Protein Degradation. *PNAS*, 101: 9085-9090, 2004

Koepp D.M. *et al.*, Phosphorylation-Dependent Ubiquitination of Cyclin E by the SCF^{Fbw7} Ubiquitin Ligase. *Science*, 294: 173-177, 2001

Reviewed and approved by site quality representative.

Unless otherwise stated in our catalogue or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses or any type of consumption or application to humans or animals.

© 2014 Eurofins Pharma Discovery Services UK Limited is an independent member of Eurofins Discovery Services.