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Certificate of Analysis

$SCF^{Skp2/Cks1}$ complex, active

(Recombinant E3 ligase expressed in Sf21 insect cells)
Item # 23-023, 23-023-K, 23-023M
Parent Lot # D11EP030N

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 6His-tagged, recombinant human Skp2 full length, *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 and complexed *in vitro* with *C*-terminal 6His-tagged, recombinant human Cks1 full length expressed in *E.coli* and purified using immobilized metal affinity chromatography.

Purity 92.2% by SDS-PAGE and Coomassie blue staining. Cks1 MW = 11kDa Skp2 MW = 52kDa, Skp1 MW = 46kDa, Cul1 MW = 93kDa, Rbx1 MW = 12kDa

Activity (Parent lot# D11EP030N): This lot of $SCF^{Skp2/Cks1}$ complex, active is active and meets product specifications.

Formulation: 1.006mg/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.

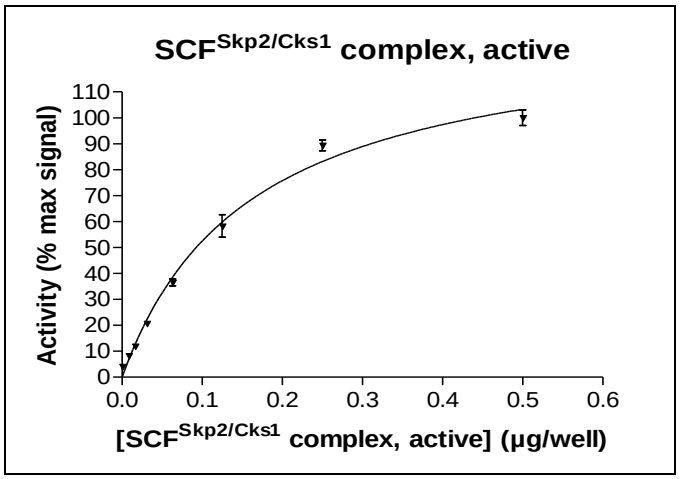
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

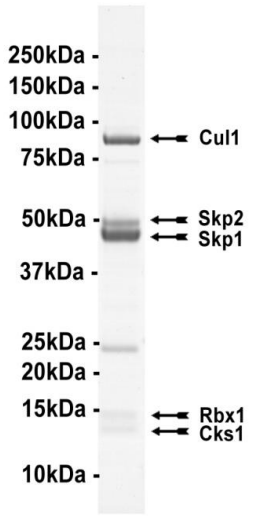
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 Skp2, Skp1, Cul1, Rbx1 and Cks1 by mass spectrometry.



SDS-PAGE and Coomassie Stain: Purity was assessed by SDS-PAGE and Coomassie blue staining using 3µg of $SCF^{Skp2/Cks1}$ complex, active.

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E3 Assay Protocol

Reagents:

1. **UBE1, active (Item # 23-021)**
2. **UbcH3, active (Item # 23-022)**
3. **$SCF^{Skp2/Cks1}$ complex, active (Item # 23-023)**
4. **p27 complex, activated (Item #. 23-024)**
5. **1x Reaction Buffer**
6. **Biotinylated-Ubiquitin**
7. **Stop Solution**

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$).

$SCF^{Skp2/Cks1}$ complex, active, is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM $MgCl_2$, 10µM ATP, 10nM UBE1, 500nM UbcH3, 20nM p27 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. $SCF^{Skp2/Cks1}$ complex activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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$SCF^{Skp2/Cks1}$ complex Information

Protein Complex of human Skp2, human Skp1, human Cul1, human Rbx1, human Cks1

Accession number GenBank NM_005983 Skp2, GenBank NM_170679 Skp1, GenBank NM_003592 Cul1, GenBank NM_014248 Rbx1, GenBank NM_001826 Cks1

Alternative Names SCF^{Skp2}, Skp2/Cks1 complex

Key Facts 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^{Skp2} mediates the ubiquitination and subsequent proteasomal degradation of target proteins involved in cell cycle progression, signal transduction and transcription. It specifically recognizes phosphorylated p27 and is involved in regulation of G1/S transition. Complex formation of SCF^{Skp2} with Cks1 is required for the degradation of p27.

Related Products Item # 23-021 UBE1, active, Item # 23-022 UbcH3, active, Item # 23-024 p27 complex, activated

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

Wang W. *et al*. A Negatively Charged Amino Acid in Skp2 Is Required for Skp2-Cks1 Interaction and Ubiquitination of $p27^{Kip1}$. J. Biol. Chem., 278: 32390-32396, 2003

Ungermannova D. *et al.* Ubiquitination of $p27^{Kip1}$ Requires Physical Interaction with Cyclin E and Probable Phosphate Recognition by SKP2. J. Biol. Chem., 280: 30301–30309, 2005

Xu S. *et al*. Substrate Recognition and Ubiquitination of $SCF^{Skp2/Cks1}$ Ubiquitin-Protein Isopeptide Ligase. J. Biol. Chem., 282: 15462–15470, 2007

Xu K. *et al*. Protein–Protein Interactions Involved in the Recognition of p27 by E3 Ubiquitin Ligase. Biochem. J. 371: 957-964, 2003

Reviewed and approved by site quality representative.

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