

Certificate of Analysis

VHL complex, active

(Recombinant E3 ligase expressed in Sf21 insect cells)

Item # 23-044, 23-044-K, 23-044M

Parent Lot # WAD0380

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 VHL amino acids 54-end, *N*-terminal GST tagged, recombinant human Elongin C, untagged, recombinant human Elongin B, *N*-terminal 6His tagged, recombinant human Cul2 and untagged recombinant human Rbx1 co-expressed by baculovirus in Sf21 insect cells. Purified using glutathione sepharose.

Combined Purity 86% by SDS-PAGE and Coomassie blue staining. VHL MW = 45kDa, Elongin C MW = 40kDa, Elongin B MW = 13kDa, Cul2 MW = 91kDa, Rbx1 MW = 12kDa

Activity (Parent lot# WAD0380): This lot of VHL complex, active is active and meets product specifications.

Formulation: 0.68mg/ml of enzyme in 50mM Tris/HCl pH7.5, 150mM NaCl, 0.1mM EGTA, 0.03% Brij-35, 270mM sucrose, 1mM benzamidine, 0.2mM PMSF, 0.1% 2-mercaptoethanol. Frozen solution.

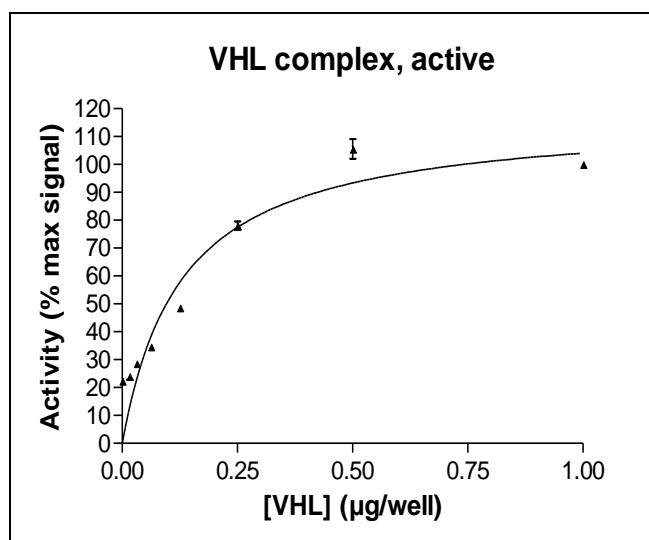
Storage and Stability: On receipt of material store at -70°C. Unopened reagent is stable for a minimum of 1 year from date of shipment when stored at recommended storage temperature. Avoid repeat freeze/thaw cycles. For maximum recovery of product, centrifuge original vial prior to removing the cap.

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.

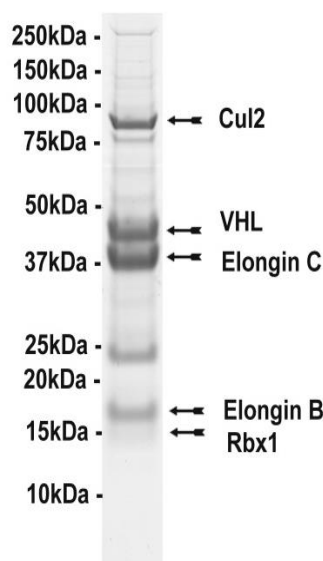
FOR IN VITRO RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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 VHL, Elongin C, Elongin B, Cul2 and Rbx1 by mass spectrometry.



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

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E3 Assay Protocol

Reagents:

- | | |
|--|---------------------------|
| 1. UBE1, active (Item # 23-021) | 5. 1x Reaction Buffer |
| 2. UbchH4, active (Item # 23-025) | 6. Biotinylated-Ubiquitin |
| 3. VHL complex, active (Item # 23-044) | 7. Stop Solution |
| 4. HIF-1 α (Item # 23-045) | |

Assay Outline:

All enzymes and reagents are diluted in the 1x reaction buffer (25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂).

VHL complex, active is incubated with 25mM MOPS pH7.5, 0.01% Tween 20, 5mM MgCl₂, 10 μ M ATP, 10nM UBE1, 1 μ M UbchH4, 50nM HIF-1 α , and 2 μ M 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. VHL complex activity is measured by detection of bound ubiquitin via electrochemiluminescence.

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VHL complex Information

Protein Complex of human VHL, human Elongin C, human Elongin B, human Cul2, human Rbx1

Accession number GenBank NM_000551 VHL, GenBank NM_005648 Elongin C, GenBank NM_007108 Elongin B, GenBank NM_003591 Cul2, GenBank NM_014248 Rbx1

Alternative Names VCB-Cul2 complex, ECV complex, CBC^{VHL}

Key Facts The VHL complex is a multi-subunit ubiquitin ligase composed of VHL, Elongin B, Elongin C, Cul2 and Rbx1. The VHL protein serves as the substrate recognition component and is linked by Elongin C to a heterodimeric Cul2/Rbx1 module that functions as a potent activator of the ubiquitination of target proteins by an E2 conjugating enzyme. Elongin B interacts with the complex through Elongin C and appears to stabilize the binding of Elongin C to VHL. The primary function of the VHL complex is to regulate HIF (hypoxia inducible factor) activity by targeting the hydroxylated HIF-1 α subunit for ubiquitination and rapid proteasomal degradation under normoxic conditions. It therefore plays an important role in the regulation of hypoxia-inducible genes such as the vascular endothelial growth factor (VEGF) and glucose transport-1 (Glut-1). Mutations in VHL are associated with the inherited von Hippel-Lindau (VHL) cancer syndrome and numerous forms of renal cell carcinoma.

Related Products Item # 23-021 UBE1, active, Item # 23-025 Ubch4, active, Item # 23-029 Ubch5a, active, Item # 23-035 Ubch5c, active, Item # 23-045 HIF-1 α

Selected References

Tanimoto K. *et al.*, Mechanism of Regulation of the Hypoxia-Inducible Factor-1 alpha by the von Hippel-Lindau Tumor Suppressor Protein. EMBO J. 19: 4298-4309, 2000

Stebbins C. E. *et al.*, Structure of the VHL-ElonginC-ElonginB Complex: Implications for VHL Tumor Suppressor Function. Science, 284: 455-461, 1999

Tyers M. and Rottapel R. VHL: A Very Hip Ligase. PNAS, 96: 12230–12232, 1999

Kamura T. *et al.*, Activation of HIF1a Ubiquitination by a Reconstituted von Hippel-Lindau (VHL) Tumor Suppressor Complex. PNAS, 97: 10430-10435, 2000

Kim W.Y. and Kaelin W.G. Role of VHL Gene Mutation in Human Cancer. J Clin Oncol. 22: 4991-5004, 2004

Reviewed and approved by site quality representative.

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