

## VHL/ELOB/ELOC/CUL2/RBX1 Complex

Recombinant full-length human proteins expressed in Sf9 cells

Catalog # **U272-380H**

Lot # E4404-3

### Product Description

Recombinant full-length human VHL, ELOB, ELOC, CUL2, and RBX1 were expressed by baculovirus in Sf9 insect cells using N-terminal His-tags for all proteins. The gene accession numbers are:

- **VHL:** [NM\\_000551.3](#)
- **ELOB:** [NM\\_007108.3](#)
- **ELOC:** [NM\\_001204857.1](#)
- **CUL2:** [NM\\_001198777.1](#)
- **RBX1:** [NM\\_014248.3](#)

### Alternative Name(s)

- **VHL:** von Hippel-Lindau disease tumor suppressor; protein G7; pVHL; HRCA1; RCA1; VHL1
- **ELOB:** Elongin-B; TCEB2; SIII p18; RNA polymerase II transcription factor SIII subunit B
- **ELOC:** Elongin-C; TCEB1; SIII; RNA polymerase II transcription factor SIII subunit C
- **CUL2:** Cullin-2; MGC131970
- **RBX1:** RING-box protein 1; E3 ubiquitin-protein ligase RBX1; Protein YYP

### Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.25mM DTT, 25% glycerol.

### Storage and Stability

Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

### Scientific Background

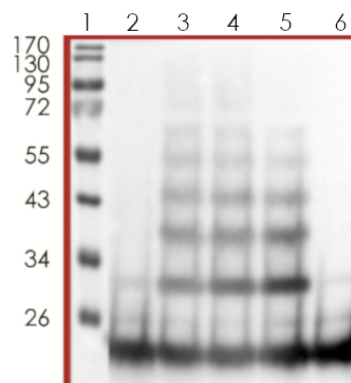
The VHL/ELOB/ELOC/CUL2/RBX1 complex, also known as the VBC complex, is an E3 ubiquitin ligase that plays a critical role in the regulation of the hypoxia-inducible factor (HIF) pathway. HIF is a transcription factor that is activated in response to low oxygen levels and regulates the expression of genes involved in angiogenesis, erythropoiesis, and metabolism. Under normoxic conditions, HIF is targeted for degradation by the VBC complex, which recognizes and ubiquitinates HIF for proteasomal degradation. Dysregulation of the HIF pathway is also a hallmark of many cancers and targeting the VBC complex has emerged as a promising therapeutic strategy for the treatment of cancer.

### References

1. Ohh M.: Ubiquitin pathway in VHL cancer syndrome. *Cancer Cell*. 10(2):77-78, 2006.
2. Min, J.H. et al: Structure of an HIF-1alpha-pVHL complex: hydroxyproline recognition in signaling. *Science*. 296(5574):1886-1889, 2002.

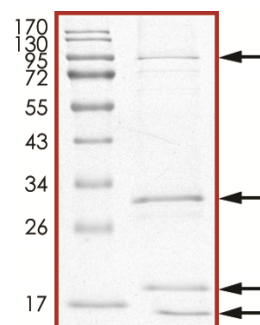
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### Activity



PROTAC-mediated ubiquitin reaction of KRAS (G12D) was used to confirm VHL E3 ubiquitin ligase activity. Lane 1: Marker. Lane 2: 800ng VHL Complex. Lane 3: 0.25µM PROTAC + VHL Complex 800ng. Lane 4: 0.5µM PROTAC + VHL Complex 800ng. Lane 5: 1µM PROTAC + VHL Complex 800ng. Lane 6: 1µM PROTAC. Primary antibody: Rabbit Anti-KRAS (G12D) (1:1,000). Secondary antibody: Anti-Rabbit HRP (1:10,000). Exposure time: 3s.

### Purity



The purity of VHL/ELOB/ELOC/CUL2/RBX1 was determined to be >90% by densitometry.

Subunit	Observed MW (~kDa)	Calculated MW (~kDa)
VHL	29	24
ELOB	18	13.4
ELOC	14	12.7
CUL2	94	85
RBX1	14	12.3

## VHL/ELOB/ELOC/CUL2/RBX1 Complex

Recombinant full-length human proteins expressed in Sf9 cells

Catalog #	U272-380H
Lot #	E4404-3
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

# Activity Assay Protocol

## Reaction Components

### Active Enzymes

Active VHL/ELOB/ELOC/CUL2/RBX1 complex (Catalog #: U272-380H), UBA1 (Catalog #: U201-380G) and UBE2D2 (Catalog #: U214-380H).

### 5X Ubiquitination Buffer

Buffer components: 0.2 M Tris-HCl (pH7.5), 50mM MgCl<sub>2</sub>, 0.5 mg/mL BSA. Add 1 mM DTT prior to use.

### PROTAC

Active KRAS(G12D) degrader comprising a binding moiety for VHL E3 ubiquitin ligase

### Substrates

Recombinant human KRAS(G12D) (Catalog #: R06-32BH)  
Recombinant Ubiquitin (Catalog #: U06-54N)  
Ultra Pure ATP (Promega V915)

### Antibodies

Rabbit anti KRAS(G12D) (Cell Signaling #14429)  
Anti rabbit HRP (Catalog #: G33-62G)

## Assay Protocol

The VHL E3 ubiquitin ligase assay is performed by setting up a PROTAC-mediated ubiquitinating reaction of KRAS(G12D), followed by immunoblotting the protein target.

- Step 1.** Thaw the active VHL complex, UBA1, UBE2D2, ubiquitin and KRAS(G12D) on ice, equilibrate all other reaction components except the antibodies to room temperature.
- Step 2.** For EACH reaction, prepare a master mix containing the following components diluted with 1X Ubiquitination Buffer:
  - o 2 µl of 0.1 µg/µl UBA1
  - o 1 µl of 0.7 µg/µl UBE2D2
  - o 2 µl of 2 µg/µl Ubiquitin
  - o 2 µl of 1.2 µg/µl KRAS(G12D)
- Step 3.** Sequentially dispense the following working solutions to microcentrifuge tubes with locking snap cap.
  - 1) 7 µl master mix
  - 2) 1 µl of 5 µM degrader
  - 3) 8 µl of VHL complex at designated concentration (starting at 0.1 µg/µl)

Note: Replace component with the buffer for appropriate controls.
- Step 4.** Dispense 4 µl of 0.5 mM ATP to all tubes to start the reaction. This gives a final volume of 20 µl for each reaction.
- Step 5.** Gently tap each tube to mix and incubate at 37°C for 1 hour. When complete, store samples on ice (or frozen) before proceeding to next step.
- Step 6.** Run standard Western Blotting protocol for all samples using rabbit anti-KRAS(G12D) (1:1000) and anti-rabbit HRP (1:10000).

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# SAFETY DATA SHEET

## Article 1 – Product Identification

**Product Name: VHL/ELOB/ELOC/CUL2/RBX1 Complex**

**Catalog # U272-380H**

*This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.*

Manufacturer's Name: SignalChem Biotech Inc.  
 Street Address: 110-13120 Vanier Place  
 City, Prov. Postal Code: Richmond, BC, V6V 2J2  
 Fax: 604-232-4601  
 EMERGENCY PHONE: 604-232-4600

## Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

## Article 3 – Composition/Information on Ingredients

**Chemical Characterization:** Mixtures.

**Description:** This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	25%
NaCl	Sodium chloride	7647-14-5	1.75%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%

## Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breath, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

## Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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## Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

## Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

## Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**  
Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	USA

- **Appropriate engineering controls:**  
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**  
**Respiratory protection:**  
Use appropriate respirator if there is inadequate ventilation by following the government standards.  
**Hand protection:**  
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.  
**Eye/face protection:**  
Safety goggles with side-shields approved under appropriate government standards.  
**Skin/body protection:**  
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

## Article 9 – Physical and Chemical Properties

<b>Appearance:</b> Colorless fluid.	<b>Danger of explosion:</b> Product does not present an explosion hazard.
<b>Odour/Odour Threshold:</b> Not determined.	<b>Explosion limits:</b> Lower: 0.9 Vol %; Upper: 0.0 Vol %.
<b>pH:</b> Not available.	<b>Decomposition temperature:</b> Not available.
<b>Melting point/freezing point:</b> Not determined.	<b>Vapor pressure at 20 °C:</b> 0.1 hPa
<b>Boiling point/Boiling range:</b> 100 °C.	<b>Density:</b> Not determined.
<b>Flash point:</b> > 100 °C.	<b>Relative density:</b> Not determined.
<b>Flammability (solid, gaseous):</b> Not determined.	<b>Vapor density:</b> Not determined.
<b>Ignition temperature:</b> 400 °C.	<b>Evaporation rate:</b> Not determined.
<b>Auto-igniting:</b> Product is not self-igniting.	<b>Solubility in / Miscibility with Water:</b> Fully miscible.

## Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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## Article 11 - Toxicological Information

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- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  - Inhalation:** May be harmful if inhaled. May cause respiratory tract irritation.
  - Ingestion:** May be harmful if swallowed.
  - Skin:** May be harmful if absorbed through skin. May cause skin irritation.
  - Eyes:** May cause eye irritation.
- **Signs and Symptoms of Exposure:**
  - Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

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## Article 12 - Ecological Information

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- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

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## Article 13 - Disposal Considerations

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- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

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## Article 14 - Transport Information

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- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

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## Article 15 - Regulatory Information

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- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

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## Article 16 - Other Information

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The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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