

CRBN/DDB1/CUL4A/RBX1 Complex

Recombinant full-length human proteins expressed in Sf9 cells

Catalog # U273-380H

Lot # U4754-18

Product Description

Recombinant full-length human CRBN, DDB1, CUL4A, and RBX1 were expressed by baculovirus in Sf9 insect cells using N-terminal His-tags for all proteins. The protein accession numbers are:

- **CRBN:** [Q96SW2](#)
- **DDB1:** [Q16531](#)
- **CUL4A:** [Q13619](#)
- **RBX1:** [P62877](#)

Alternative Name(s)

- **CRBN:** MRT2; MRT2A; protein cereblon
- **DDB1:** DDBA; UV-DDB1; WHIKERS; XAP1; XPCE; XPE; DNA damage-binding protein 1
- **CUL4A:** cullin-4A; CUL-4A
- **RBX1:** RING-box protein 1; E3 ubiquitin-protein ligase RBX1; Protein ZYP

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.

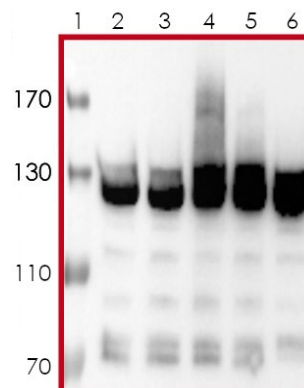
Scientific Background

The CRBN/DDB1/CUL4A/RBX1 complex, also known as CRL4 CRBN E3 ubiquitin ligase complex, forms an E3 ubiquitin ligase that tags target proteins for ubiquitin-mediated degradation, thereby regulating various cellular processes such as cell cycle progression, DNA repair, and protein homeostasis. CRBN is a substrate receptor that serves as a binding site for specific target proteins, allowing the complex to recognize and ubiquitinate them for degradation. DDB1 acts as an adaptor protein that connects CRBN to CUL4A within the complex. CUL4 acts as a scaffold within the complex, providing the structural backbone for the assembly of the complex and facilitates the transfer of ubiquitin molecules to the target proteins. RBX1 interacts with CUL4A and facilitates the transfer of ubiquitin moieties to the target proteins, marking them for degradation by the proteasome.

References

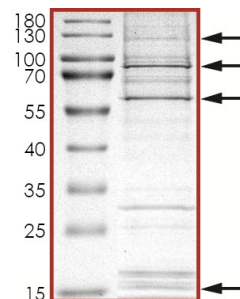
1. Ito T, et al. (2010). Identification of a primary target of thalidomide teratogenicity. *Science*, 327(5971), 1345-1350.
2. Sugawara K, et al. (2005). A multistep damage recognition mechanism for global genomic nucleotide excision repair. *Genes & Development*, 19(17), 2192-2203.

Activity



PROTAC-mediated ubiquitin reaction of BRD3 was used to confirm CRBN E3 ubiquitin ligase activity. Lane 1: Marker. Lane 2: CRBN Complex + BRD3 + Ub. Lane 3: 0.1µM PROTAC + CRBN Complex + BRD3 + Ub. Lane 4: 0.4µM PROTAC + CRBN Complex + BRD3 + Ub. Lane 5: 2µM PROTAC + CRBN Complex + BRD3 + Ub. Lane 6: 0.4µM PROTAC + BRD3 + Ub. Primary antibody: Mouse anti-BRD3 (1:200). Secondary antibody: Anti-Mouse HRP (1:10,000). Exposure time: 30s.

Purity



The purity of CRBN/DDB1/CUL4A/RBX1 was determined to be **>70%** by densitometry.

Subunit	Observed MW (~kDa)	Calculated MW (~kDa)
CRBN	58	51
DDB1	128	131
CUL4A	88	87
RBX1	14	12

CRBN/DDB1/CUL4A/RBX1 Complex

Recombinant full-length human proteins expressed in Sf9 cells

Catalog #	U273-380H
Lot #	U4754-18
Purity	>70%
Concentration	0.05 µg/ul
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.

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Activity Assay Protocol

Reaction Components

Enzymes

CRBN/DDB1/CUL4A/RBX1 complex (Catalog #: U273-380H)
UBA1 (Catalog #: U201-380G)
UBE2D3 (Catalog #: U215-30H)
UBE2G1 (Catalog #: U221-30H)

5X CRBN Buffer

Buffer components: 0.1 M HEPES (pH7.5), 0.5 M NaCl, 50 mM MgCl₂. Add 5 mM DTT prior to use.

PROTAC

dBET1 dissolved in DMSO at 10 mM stock concentration.

Substrates

Recombinant human BRD3 (Catalog #: B17-30G)
Recombinant Ubiquitin (Catalog #: U06-54N)
Ultra Pure ATP (Promega V915)

Antibodies

Mouse monoclonal IgG1 BRD3 antibody (SCBT: sc-81202)
Anti-mouse HRP (Catalog #: G32-62G)

Assay Protocol

The Cereblon E3 ubiquitin ligase assay is performed by setting up a PROTAC-mediated ubiquitinating reaction of recombinant BRD3 protein, followed by immunoblotting the protein target.

- Step 1.** Thaw the active CRBN complex, UBA1, UBE2D3, UBE2G1, ubiquitin and BRD3 on ice, equilibrate all other reaction components to room temperature.
- Step 2.** For EACH reaction, prepare a **cocktail A** containing the following components diluted with 1X CRBN Buffer:
 - o 5 µl of 0.1 µg/µl UBA1
 - o 1.25 µl of 0.6 µg/µl UBE2D3
 - o 2 µl of 0.5 µg/µl UBE2G1
 - o 2.5 µl of 2 µg/µl Ubiquitin
 - o 1.25 µl of 10 mM ATP
- Step 3.** Incubate cocktail A at room temperature for 30 minutes.
- Step 4.** For EACH reaction, prepare a **cocktail B** containing the following components diluted with 1X CRBN Buffer:
 - o 5 µl of 0.2 µg/µl BRD3
 - o 6.5 µl of 0.05 µg/µl CRBN complex
 - o 1 µl of 8 µM dBET1

Note: Replace component with the buffer for appropriate controls.
- Step 5.** Incubate cocktail B at room temperature for 5 minutes, then dispense 12.5 µl each of **cocktail A** and **B** in a microcentrifuge tube with locking snap cap.
- Step 6.** Gently tap tube to mix and incubate at 37°C for 2 hours. When complete, briefly spin down the tubes and store samples on ice (or frozen) until next step.
- Step 7.** Run standard Western Blotting protocol for all samples using mouse anti-BRD3 (1:200) and anti-mouse HRP (1:10000).

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: CRBN/DDB1/CUL4A/RBX1 Complex

Catalog # U273-380H

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 ³ for mist	British Columbia, Canada
TWA	3 mg/m ³ for respirable mist	British Columbia, Canada
TWA	10 mg/m ³	Alberta, Canada
TWAEV	10 mg/m ³	Ontario, Canada
TWAEV	10 mg/m ³	Quebec, Canada
TWA	10 mg/m ³	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

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

- **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.

Article 12 - Ecological Information

- **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.

Article 13 - Disposal Considerations

- **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.

Article 14 - Transport Information

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

Article 15 - Regulatory Information

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

Article 16 - Other Information

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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