

BRD4 (BD1), Active

Recombinant human protein expressed in *E. coli* cells

Catalog # **B18-31H**

Lot # E4403-5

Product Description

Recombinant human BRD4 (BD1) (49-170) was expressed in *E. coli* cells using an N-terminal His tag. The gene accession number is [NM_014299](#).

Alternative Name(s)

Bromodomain containing 4, BRD4, HUNK1, MCAP

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

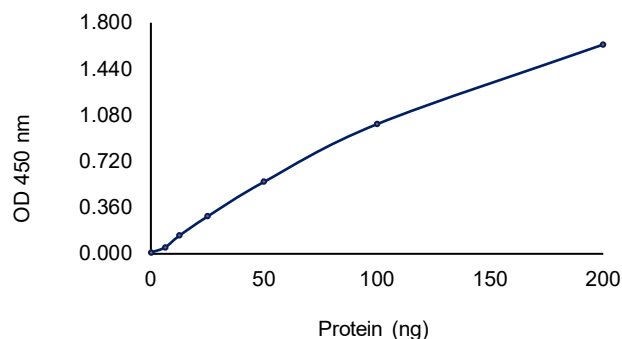
Bromodomain-containing protein 4 (BRD4) is a member of the bromodomain and extra-terminal domain (BET) protein family. BRD4 contains two tandem bromodomains: BD1 and BD2. BRD4 (BD1) functions as an epigenetic reader – recognizing and interpreting histone acetylation marks to recruit transcriptional machinery and chromatin remodeling complexes. BRD4 (BD1) is involved in the regulation of cell cycle progression, with its overexpression promoting cell proliferation and survival. Furthermore, it has been implicated in the development and progression of cancer as it regulates the expression of oncogenes and tumor suppressor genes.

References

1. Wu T, Pinto HB, Kamikawa YF, et al. The BRD4 BD1-BD2 interface regulates transcriptional output of BET inhibitors. *Nat Chem Biol.* 2020;16(6):619-627. doi:10.1038/s41589-020-0522-0.
2. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature.* 2010;468(7327):1067-1073. doi:10.1038/nature09504.
3. Shi J, Wang Y, Zeng L, et al. Disrupting the interaction of BRD4 with diacetylated Twist suppresses tumorigenesis in basal-like breast cancer. *Cancer Cell.* 2014;25(2):210-225. doi:10.1016/j.ccr.2014.01.030

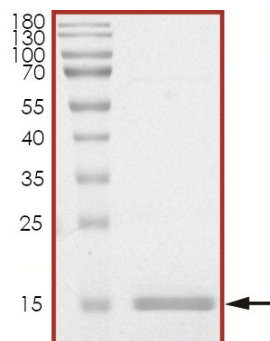
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Binding Activity



The binding activity of BRD4 (BD1) was determined to be **2,908 ng/min/mg** towards acetylated peptide substrate as per the activity assay protocol.

Purity



The purity of BRD4 (BD1) was determined to be **>90%** by densitometry.
Observed MW **~15 kDa**.
Calculated MW **~14.7 kDa**.

BRD4 (BD1), Active

Recombinant human protein expressed in *E. coli* cells

Catalog #	B18-31H
Binding Activity	2,908 ng/min/mg
Lot #	E4403-5
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

BRD4 (BD1) (Catalog #: B18-31H)

BRD4 (BD1) (0.1µg/µl) diluted with Assay Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of BRD4 (BD1) for optimal results).

Assay Dilution Buffer

BSA (Sigma, Cat#A7906) diluted to 0.5% with PBS prior to use.

Blocking Buffer

Skim milk (Medallion, Cat# X002PW0LUH) diluted to 5% with PBS prior to use.

Acetylated Assay Substrate

Acetylated assay substrate Peptide (1-21) diluted in PBS to a final concentration of 1 µg/ml.

Non-acetylated Control Substrate

Non-acetylated control substrate peptide (1-21) diluted in PBS to a final concentration of 1 µg/ml.

Standard

BRD4 (BD1) (Catalog #: B18-31H) may be used as a standard.

Detection Antibody, mouse monoclonal, biotinylated

The biotinylated detection antibody (250µg/mL) was biotinylated in-house from non-biotinylated antibody. The streptavidin HRP was from R@D Systems (Cat#: DY998). TMB substrate was from BD Biosciences (Cat#: 555214)

Assay Protocol

- Step 1.** Dilute acetylated assay substrate peptide and non-acetylated control Peptide (background) in PBS to 1 µg/ml. Dilute BRD4 (BD1) to 2 µg/ml (**Standard**), then make 2-fold dilution for 7 dilutions. Add 50µl/well of either the Acetylated assay substrate Peptide, non-acetylated control Peptide or Standard to 94 wells of a 96 well ELISA plate, leaving 2 wells without coating as **Blank** control. Incubate in 4°C overnight.
- Step 2.** Discard solution. Wash plate 3 times with ELISA Wash buffer (1X PBS with 0.05% Tween-20).
- Step 3.** Block plate with 200µl/well of 5% skim milk in PBS (**Blocking Buffer**). Incubate in 4°C overnight.
- Step 4.** Wash the plate by repeating Step 2.
- Step 5.** Thaw the BRD4 (BD1) on ice.
- Step 6.** Prepare 0.5% BSA in PBS (**Assay Dilution Buffer**). Leave on ice.
- Step 7.** Make serial dilutions of BRD4 (BD1) enzyme to the desired concentration with Assay Dilution Buffer. Leave on ice.
- Step 8.** Add the following reaction components to substrate coated wells, bringing the reaction volume up to 50µl.
 - Component 1.** 25µl of diluted BRD4 (BD1) (Catalog # B18-31H)
 - Component 2.** Optional: if testing with inhibitor. Add 25µl of inhibitor at desired concentration to 25ul enzyme solution, otherwise add 25µl Assay Dilution Buffer.
- Step 9.** Add 50µL of Assay Dilution Buffer to **Standard** wells and **Blank** control wells.
- Step 10.** Incubate the mixture at room temperature for 1 hour.
- Step 11.** Wash the plate by repeating step 2.
- Step 12.** Dilute Biotinylated detection antibody to 0.05 µg/ml in PBS with 1% BSA. Add 50µL/well to all wells and incubate at room temperature for one hour.
- Step 13.** Wash the plate by repeating step 2.
- Step 14.** Dilute streptavidin HRP in PBS with 1% BSA. Add 50µl/well to all wells and incubate at room temperature with slow shaking for 30 minutes.
- Step 15.** Discard solution and wash plate 6 times with ELISA Wash buffer.
- Step 16.** Add 50µL/well of TMB Substrate and incubate approximately 30 minutes for blue color development.
- Step 17.** Stop the reaction by adding 50µl/well of 2M sulfuric acid, turning the blue color to yellow.
- Step 18.** Read the absorbance in TECAN microplate reader at 450 nm and 570 nm.
- Step 19.** Calculate average duplicate readings for sample wells, standard wells and blank wells, if required.
- Step 20.** Generate a standard curve as OD value versus amount of standard at each concentration point. Then calculate the corresponding ng of BRD product bound to acetylated substrate from the standard curve.
- Step 21.** Calculate the BRD binding activity as outlined below.

BRD Binding Activity (ng/min/mg)

BRD Activity (ng/min/mg) = bound BRD product (ng) / (Reaction time in min) * (Enzyme amount in mg)

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: BRD4 (BD1), Active

Catalog # B18-31H

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 breathe, 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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