

## Recombinant BRD4 (44-460) protein

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**Catalog No:** 31594, 31994

**Lot No:** 26417001

**Expressed In:** *E. coli*

**Quantity:** 100, 1000 µg

**Concentration:** 1.3 µg/µl

**Source:** Human

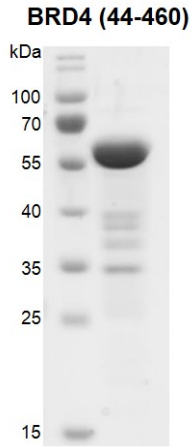
**Buffer Contents:** Recombinant BRD4 (44-460) protein is supplied at a concentration of 1.3 µg/µl in 25 mM Tris pH 8.0, 300 mM NaCl, 10% glycerol, 0.5 mM TCEP.

**Background:** Bromodomain-containing protein 4 (BRD4) belongs to the BET subclass of proteins, which are characterized by two N-terminal bromodomains and one ET (Extra Terminal) domain. BRDs associate with chromatin through their bromodomains that recognize acetylated histone lysine residues. Bromodomains function as 'readers' of these epigenetic histone marks and regulate chromatin structure and gene expression by linking associated proteins to the acetylated nucleosomal targets. The ET domain functions as a protein binding motif and exerts atypical serine-kinase activity. The BET family consists of at least four members in mouse and human, BRD2 (also referred to as FSRG1, RING3), BRD3 (FSRG2, ORFX), BRD4 (FSRG4, MCAP/HUNK1), and BRDT (FSRG3, BRD6). BRD proteins are related to the female sterile homeotic protein gene in *Drosophila*, a gene required maternally for proper expression of other homeotic genes, such as *Ubx*, which is involved in pattern formation. BRD4 has been identified recently as a therapeutic target in many cancers, including acute myeloid leukemia, multiple myeloma, Burkitt's lymphoma, NUT midline carcinoma, colon cancer, and breast cancer. BRD4 regulates the transcription of oncogenes, HIV, and human papilloma virus (HPV). It has been shown to bind and phosphorylate RNA pol II, which implicates its involvement in the regulation of eukaryotic transcription.

**Protein Details:** The protein corresponding to amino acids 44 - 460 that contains two Bromo domains of BRD4 (accession number NP\_490597.1) was expressed in *E. coli* cells and contains an N-terminal His tag and a C-terminal FLAG tag. The molecular weight of BRD4 (44-460) is 51.6 kDa. It shows binding specificity for acetylated H3K9, H3K9/ K14, H4K5, H4K8, H4K12, H4K5/K8, H4K5/K12, H4K8/K12, H4K12/K16, H4K12/K16/K20 and H4K5/K8/K12/K16, as well as acetylated RelA-K310.

**Application Notes:** This protein is suitable for use in binding assays, inhibitor screening, and selectivity profiling.

**Storage and Guarantee:** Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage. This product is for research use only and is not for use in diagnostic procedures. This product is guaranteed for 6 months from date of arrival.



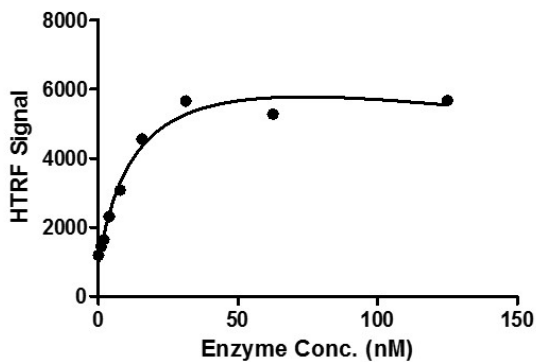
**Recombinant BRD4 (44-460) protein gel**

10% SDS-PAGE gel, stained with Coomassie blue.

MW: 51.6 kDa

Purity: > 85%

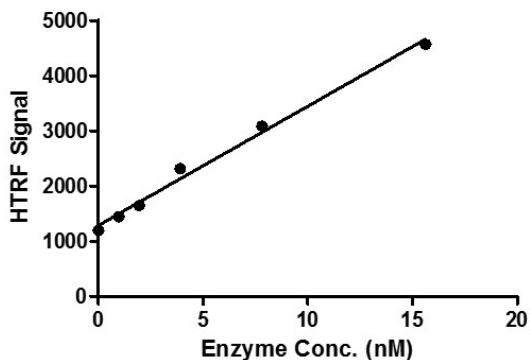
**BRD4 (44-460) Titration**



**HTRF assay for BRD4 (44-460) activity**

1  $\mu$ M H4K5/8/12/16(4ac) peptide was incubated with different concentrations of BRD4 (44-460) protein in 10  $\mu$ l reaction system containing 50 mM HEPES-NaOH pH 7.5, 0.1% BSA for 1 hour, then 10  $\mu$ l Anti-FLAG antibody and SA-XL665 mixture (each 1:100 dilution in HTRF Detection Buffer) were added to each reaction system and incubated for 30 min. All the operations and reactions were performed at room temperature.

**BRD4 (44-460) Titration**



**HTRF assay for BRD4 (44-460) activity**

1  $\mu$ M H4K5/8/12/16(4ac) peptide was incubated with different concentrations of BRD4 (44-460) protein in 10  $\mu$ l reaction system containing 50 mM HEPES-NaOH pH 7.5, 0.1% BSA for 1 hour, then 10  $\mu$ l Anti-FLAG antibody and SA-XL665 mixture (each 1:100 dilution in HTRF Detection Buffer) were added to each reaction system and incubated for 30 min. All the operations and reactions were performed at room temperature.