REACTION BIOLOGY

PRODUCT DATASHEET

BRD3 Full-Length (His)

(Bromodomain containing protein 3 (RING3L))

CATALOG NO.: RD-21-363 **LOT NO.:**

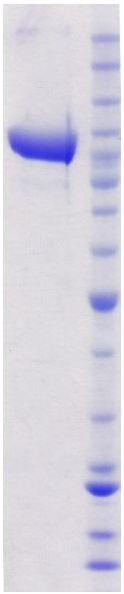
DESCRIPTION: Human recombinant BRD3, full-length construct (residues 2-726; Genbank Accession # NM_007371.3; MW = 82.8 kDa) expressed in *Sf9* insect cells with an N-terminal His-tag. BRD3, like other human members of the BET family of chromatin-binding proteins (BRD2, BRD4, BRDT), comprises two bromodomains (see reviews[1,2]), protein modules that bind ε-*N*-acetyllysine residues[3,4]. Recent results suggest an important role for BRD3 in linking acetylation of both histones and non-histone proteins to gene transcription. When overexpressed in 293 cells, BRD3, along with BRD2, binds the hyperacetylated chromatin of transcribed genes, regions enriched in acetylated histone H4 lysine-5 (H4K5Ac), H4K12Ac, H3K14Ac, but deficient in H4K16Ac and H3K9me[5]. In an *in vitro* RNA polymerase II transcription system, binding of either BRD3 or BRD2 to a chromatin template assembled with hyperacetylated histones enabled transcription through the nucleosomes[5]. In addition to acetylated histones, BRD3-1 has been found to bind the hematopoietic transcription factor GATA1 and to enhance its chromatin binding and activation of target genes[6]. Like the binding of the bromodomains-1 of BRDT and BRD4 to H4K5Ac/K8Ac, interaction of BRD3-1 with GATA1 occurs via the simultaneous binding of K312Ac and K315Ac[7]. The BET family inhibitor, I-BET151, has shown efficacy in mouse models of MLL-fusion leukemias, displacing BRD3 and BRD4 from chromatin and inhibiting transcription of genes, e.g. *BCL2*, upregulated by the MLL-fusions[8].

PURITY: >90% by SDS-PAGE

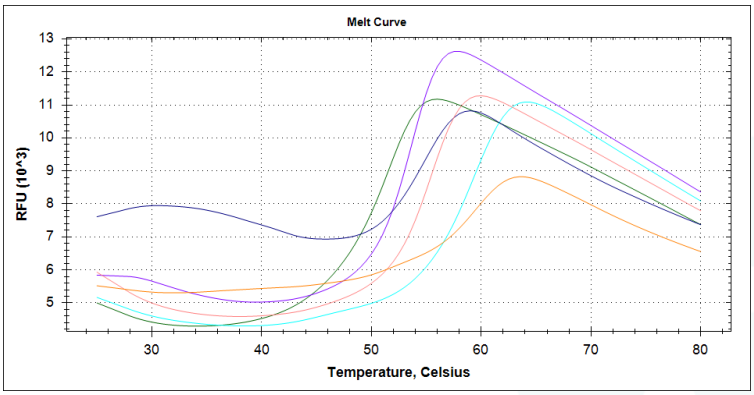
SUPPLIED AS: _ µg/µL in 50 mM Tris/HCl, pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v)

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted protein should be snap frozen, for example in a dry ice ethanol bath or liquid nitrogen. Minimize freeze/thaws if possible, but very low volume aliquots (<5 µl) or storage of diluted enzyme is not recommended.

REFERENCES: 1) B. Florence & D.V. Faller *Front. Biosci.* 2001 **6** D1008; 2) S.-Y. Wu & C.-M. Chiang *J. Biol. Chem.* 2007 **282** 13141; 3) D.J. Owen *et al. EMBO J.* 2000 **19** 6141; 4)) L. Zeng & M.-M. Zhou *FEBS Lett.* 2002 **513** 124; 5) G. LeRoy *et al. Mol. Cell* 2008 **30** 51; 6) J.M. Lamonica *et al. Proc. Natl. Acad. Sci. USA* 2011 **108** E159; 7) R. Gamsjaeger *et al. Mol. Cell. Biol.* 2011 **31** 2632; 8) M.A. Dawson *et al. Nature* 2011 **478** 529



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of RBC BRD3 Full-Length (His). MW markers (right lane) are, from top, 220, 160, 120, 100, 90, 80, 70, 60, 50, 40, 30, 25, 20, 15, 10 kDa.



Differential Scanning Fluorimetry of RBC BRD3 Full-Length (His) in the Absence or Presence of Several Inhibitors. Thermal denaturation of BRD3 Full-Length (His) is detected (CFX384™ Touch thermal cycler, 'FRET' channel; Bio-Rad) by increased binding and fluorescence of the dye SYPRO® Orange (Life Technologies). Addition of a BET bromodomain inhibitor/ligand—BET151, (+)-JQ1, Bromosporine, PFI-1, or RVX-208 (all 25 µM)—stabilizes the protein folding and shifts the T_m (inflection point) from 51.5°C (DMSO control) to 59.5°C, 59.5°C, 55°C, 55.5°C, or 53.5°C respectively.

Reaction Biology
1 Great Valley Parkway, Malvern PA, USA 19355
requests@reactionbiology.com www.reactionbiology.com