REACTION BIOLOGY

PRODUCT DATASHEET

BRD4 (Bromodomain containing protein 4, long isoform), Full Length

CATALOG NO.: RD-21-153 **LOT NO.:**

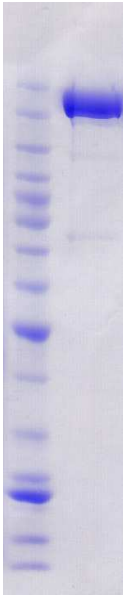
DESCRIPTION: Human recombinant BRD4, (residues 2-1362; long isoform, Genbank Accession # NM_058243; MW = 156.5 kDa) expressed in *Sf9* insect cells with an N-terminal His-tag and C-terminal StrepII-tag. BRD4, like other human members of the BET family of chromatin-binding proteins (BRD2, BRD3, BRDT), comprises two bromodomains (see reviews[1,2]), protein modules that bind ε-*N*-acetyllysine residues[3,4]. The ubiquitously expressed BRD4 functions as a transcriptional regulator[2] with roles in cell cycle progression[5,6] and has recently been shown to be an atypical kinase that can phosphorylate RNA Pol II[7]. Recent structural studies have shown that BRD4-1[8], like the bromodomain-1 of fellow BET family protein BRDT[9], can bind simultaneously to two acetyllysine residues with appropriate spacing and sequence context, for example a histone H4 peptide acetylated at lysines 5 and 8 (H4K5AcK8Ac)[8]. Chromosomal translocations that produce BRD4-NUT fusion proteins are implicated in causation of a rare and aggressive cancer, NUT midline carcinoma[10]. Selective inhibitors of BRD4/BET family bromodomains[11-13] are showing promise as possible therapeutic agents for cancer[11,14-16] and inflammation[12].

PURITY: >85% by SDS-PAGE

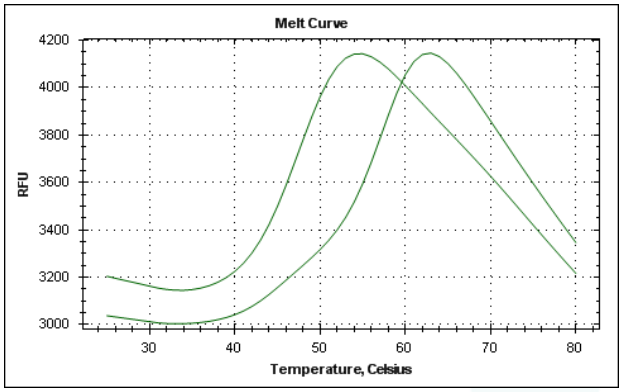
SUPPLIED AS: _ µg/µL in 50 mM Tris/HCl, pH 8.0, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) as determined by OD_{280}

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) A. Dey *et al. Mol. Cell. Biol.* 2000 **20** 6537; 6) T. Maruyama *et al. Mol. Cell. Biol.* 2002 **22** 6509; 7) B.N. Devaiah *et al. Proc. Natl. Acad. Sci. USA* 2012 **109** 6927; 8) P. Filippakopoulos *et al. Cell* 2012 **149** 214; 9) J. Morinière *et al. Nature* 2009 **461** 664; 10) C.A. French *J. Clin. Pathol.* 2010 **63** 492; 11) P. Filippakopoulos *et al. Nature* 2010 **468** 1067; 12) E. Nicodeme *et al. Nature* 2010 **468** 1119; 13) D.S. Hewings *et al. J. Med. Chem.* 2012 **55** 9393; 14) J.E. Delmore *et al. Cell* 2011 **146** 904; 15) J. Zuber *et al. Nature* 2011 **478** 524; 16) W.W. Lockwood *et al. Proc. Natl. Acad. Sci. USA* 2012 **109** 19408



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg of RBC BRD4, Full Length. MW markers (left 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 BRD4,-Full Length in Presence or Absence of (+)-JQ1. Thermal denaturation of BRD4, Full Length (1 mg/mL) is detected (CFX384™ Touch thermal cycler, 'FRET' channel; Bio-Rad) by increased binding and fluorescence of the dye SYPRO® Orange (Life Technologies). Addition of the BET bromodomain inhibitor/ligand (+)-JQ1 (10 µM) stabilizes the protein folding and shifts the T_m (inflection point) from 47°C to 57°C.

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