

BRD4 (Bromodomain containing protein 4), (aa 1-744)

**CATALOG NO.:** RD-21-427

**LOT NO.:**

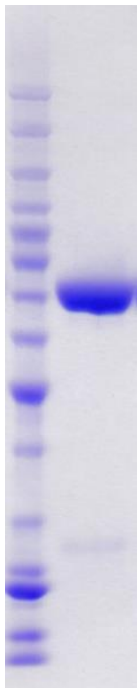
**DESCRIPTION:** Human recombinant BRD4, (residues 1-744; Genbank Accession # NM\_058243; MW = 85.7 kDa) expressed in Sf9 insect cells with an N-terminal StrepII-tag and C-terminal His-tag. BRD4, like other human members of the BET family of chromatin-binding proteins (BRD2, BRD3, BRDT), comprises two bromodomains (see reviews<sup>1,2</sup>), protein modules that bind  $\epsilon$ -N-acetyllysine residues<sup>3,4</sup>. The ubiquitously expressed BRD4 functions as a transcriptional regulator<sup>2</sup> with roles in cell cycle progression<sup>5,6</sup> and has recently been shown to be an atypical kinase that can phosphorylate RNA Pol II<sup>7</sup>. Recent structural studies have shown that BRD4-1<sup>8</sup>, like the bromodomain-1 of fellow BET family protein BRDT<sup>9</sup>, 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)<sup>8</sup>. Chromosomal translocations that produce BRD4-NUT fusion proteins are implicated in causation of a rare and aggressive cancer, NUT midline carcinoma<sup>10</sup>. Selective inhibitors of BRD4/BET family bromodomains<sup>11-13</sup> are showing promise as possible therapeutic agents for cancer<sup>11,14-16</sup> and inflammation<sup>12</sup>.

**PURITY:** >90% by SDS-PAGE

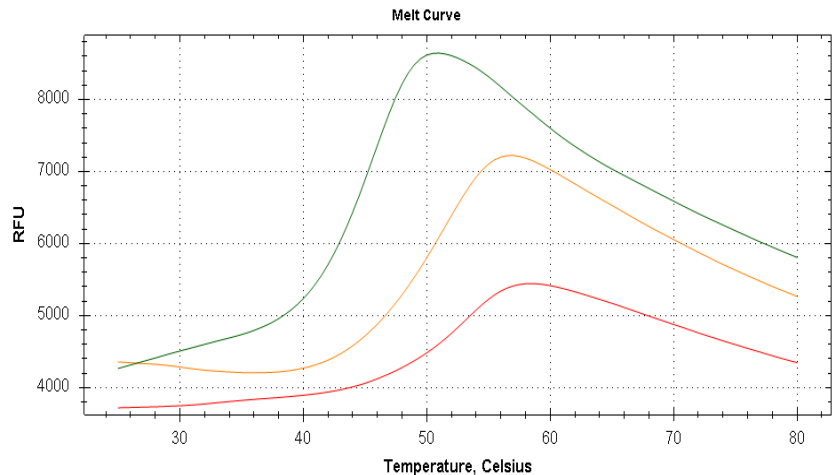
**SUPPLIED AS:** \_  $\mu$ g/ $\mu$ L in 50 mM Tris/HCl, pH 8, 500 mM NaCl, 10% glycerol (v/v).

**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  $\mu$ l) 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  $\mu$ g of RBC BRD4 (1-744).** MW markers (flanking lanes) 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 (1-744) in Absence or Presence of Several Inhibitors.** Thermal denaturation of BRD4 (1-744) 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 or iBET151 (both at 10  $\mu$ M) stabilizes the protein folding and shifts the  $T_m$  (inflection point) from 45.5°C (DMSO control) to 53°C and 51.5°C, respectively.

This product is NOT intended for therapeutic or diagnostic use in animals or in humans.