REACTION BIOLOGY

PRODUCT DATASHEET

EP300 (His) (E1A-associated protein p300; p300)

CATALOG NO.: RD-11-222 **LOT NO.:**

DESCRIPTION: Human recombinant EP300 bromodomain (residues 1040-1161; Genbank Accession # NM_001429 MW = 17.2 kDa) expressed in *E. coli* with an N-terminal His-tag. EP300 and the closely related CREBBP (CBP) are major regulators of gene transcription (co-activators) and function as lysine acetyltransferases for histones (i.e. HATs) and for numerous other proteins (e.g. p53). Aside from the acetyllysine-binding bromodomain and the catalytic HAT domain, native EP300 comprises multiple protein-protein interaction domains (3 Cys-His-rich (CH) domains, KIX domain, steroid receptor co-activator interaction domain (SID)) and also has E3 and E4 ubiquitin ligase activities. (See reviews and references therein[1,2].) The EP300 bromodomain binds various Lys(Ac) residues in singly acetylated histone peptide microarrays (histones H1.4, H2A, H3, H4) with binding to histone H3 K56(Ac) and H4 K44(Ac) confirmed in solution by isothermal titration calorimetry (ITC)[3]. A crystal structure of the EP300 bromodomain has been determined (MMDB ID: 75689; PDB ID: 3I3J)[3]. Selective inhibitors of the CREBBP/p300 bromodomains have been developed (SGC-CBP30, I-CBP112; see figure below).

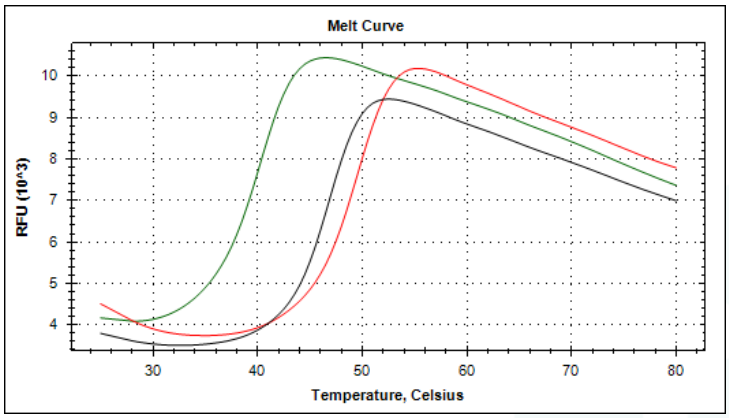
PURITY: >90% by SDS-PAGE

SUPPLIED AS: _ µg/µL in 50 mM Tris HCl, pH 7.5, 500 mM NaCl, 1 mM TCEP, 10% glycerol (v/v) 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 protein is not recommended.

REFERENCES: 1) L. Wang *et al. Curr. Opin. Struct. Biol.* 2008 **18** 741; 2) P.-H. Holmqvist & M. Mannervik *Transcription* 2013 **4** 18; 3) P. Filippakopoulos *et al. Cell* 2012 **149** 214

Coomassie blue-stained SDS-PAGE (4-12% acrylamide) of 4 µg of RBC EP300 (His). MW markers (left) are, from top: 220, 160, 120, 100, 90, 80, 70, 60, **50**, 40, 30, 25, **20**, 15 & 10 kDa.



Differential Scanning Fluorimetry of RBC EP300 (His) in the presence or absence of bromodomain ligands. Thermal denaturation of EP300 (His) is detected (CFX384 TMTouch thermal cycler, 'FRET' channel; Bio-Rad) by increased binding and fluorescence of the dye SYPRO®Orange (Life Technologies). Addition of 25 µM CBP112 (black) or SGC-CBP30 (red) stabilizes the protein folding and shifts the Tm (inflection point) from 40.5°C to 47°C or 49.5°C, respectively.

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