REACTION BIOLOGY

PRODUCT DATASHEET

$WRAD_2$ Complex (WDR5, RbBP5, Ash2L, $(DPY\text{-}30)_2$)

CATALOG NO.: HMT-14-109 **LOT NO.:**

DESCRIPTION: Human recombinants WDR5 (22-334; NM_017588; 34 kDa), RbBP5 (1-538; NM_005057; 61 kDa), Ash2L (2-534; NM_001105214; 63 kDa) and DPY-30 (1-99; NM_0325742; 13 kDa; two per complex). All proteins were expressed in *E. coli* with N-terminal His-tags. Catalyzes the transfer of methyl groups from S-adenosyl-L-methionine (SAM) to the ε-amino function of protein L-lysine residues, specifically lysine-4 of histone H3 (H3K4). Members of the $WRAD_2$ complex are found in the active, assembled histone H3K4 methyltransferase complexes of all human SET1 family members, MLL1[1-3], MLL2[4], MLL3[5,6], MLL4[5,6], SET1A[6] and SET1B[6]. Binding interactions occur between WDR5 and a "WDR5 interaction motif" (*Win*)[7,8] in SET1 family histone methyltransferases, and between the subunit pairs WDR5-RbBP5, RbBP5-Ash2L and Ash2L-$(DPY\text{-}30)_2$[9]. Addition of the $WRAD_2$ subunits *in vitro* to recombinant MLL1 (3745-3969) stimulates methyltransferase activity about 600-fold[9]. Thus, $WRAD_2$ is considered an essential sub-complex of SET1 family H3K4 methyltransferases, necessary for complex assembly and full activity[2,6,9]. Moreover, recent work has shown that the $WRAD_2$ complex by itself has H3K4 methyltransferase activity, making it the first known non-SET domain/non-Dot1L lysine methyltransferase[9,10]. Interaction between MLL1 and $WRAD_2$ is required for sequential mono- and dimethylation of H3K4[9] and for methylation of nucleosomal histone H3[10]. The *Win* motif interacts with WDR5 at a site also implicated in binding the N-terminus of histone H3 and peptides based on both *Win* and H3 sequence have been found to disrupt the MLL1-WDR5 interaction and inhibit methyltransferase actiivity[7,11]. Unlike SET-domain inhibition, targeting of $WRAD_2$'s MLL regulatory interactions or its intrinsic methyltransferase activity would represent routes to MLL inhibition that would be specific to the SET1 family.

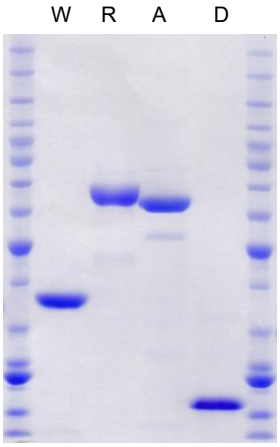
PURITY: >90% by SDS-PAGE.

ASSAY CONDITIONS: RBC's $WRAD_2$ Complex displays methyltransferase activity with an H3(1-21) peptide in a reverse phase resin binding assay. Reaction conditions are: 100 mM CHES/KOH, pH 8.5, 5 mM $MgCl_2$, 0.8 mM DTT, protein or peptide substrate, [^{3}H]-SAM.

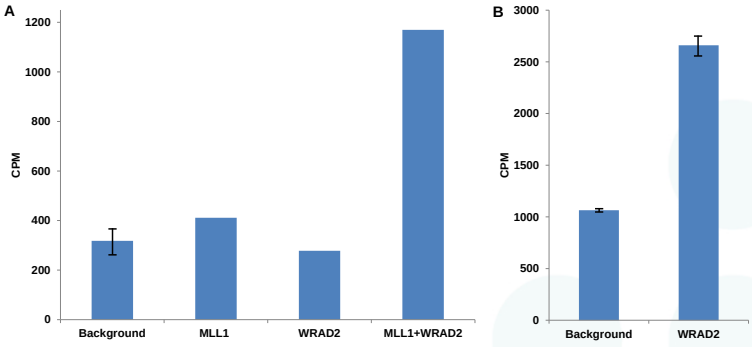
SUPPLIED AS: __ µM $WRAD_2$ Complex, as defined above, (_ µg/µl total protein) in 20 mM Tris-HCl, pH 7.5, 300 mM NaCl, 1 mM TCEP (tris(2-carboxyethyl)phosphine HCl), 10% (w/v) glycerol, 1 µM $ZnCl_2$ as determined by OD_{280}.

STORAGE: -70°C. Thaw quickly and store on ice before use. The remaining, unused, undiluted enzyme 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) A. Yokoyama *et al. Mol. Cell. Biol.* 2004 **24** 5639; 2) Y. Dou *et al. Nat. Struct. Mol. Biol.* 2006 **13** 713; 3) M.M. Steward *et al. Nat. Struct. Mol. Biol.* 2006 **13** 852; 4) R. Mo *et al. J. Biol. Chem.* 2006 **281** 15714; 5) S. Lee *et al. Proc. Natl. Acad. Sci USA* 2006 **103** 15392; 6) Y. Cho *et al. J. Biol. Chem.* 2007 **282** 20395; 7) A. Patel *et al. J. Biol. Chem.* 2008 **283** 32162; 8) J.J. Song & R.E. Kingston *J. Biol. Chem.* 2008 **283** 35258; 9) A. Patel *et al. J. Biol. Chem.* 2009 **284** 24242; 10) A. Patel *et al. J. Biol. Chem.* 2011 **286** 3359; 11) H. Karatas *et al. J. Med. Chem.* 2010 **53** 5179



Coomassie blue stained SDS-PAGE (4-12% acrylamide) of 4 µg each of the purified components of the $WRAD_2$ Complex. MW markers are from top, 220, 120, 90, 70, 60, **50**, 40, 30, **20**, 15, 10 kDa. W=WDR5; R=RbBP5; A=Ash2L; D=DPY-30. Note that His-DPY-30 (13 kDa) migrates anomalously at ~17 kDa.



$WRAD_2$ Complex Stimulates MLL1 Activity (A) and Displays Intrinsic Methyltransferase Activity (B). Reactions (10 µL) were incubated 30 min., 30°C and stopped by denaturation with guanidinium HCl. Proteins/peptides were separated from unreacted SAM by binding to a reverse phase resin (C18) and eluted for scintillation counting. Background samples were denatured prior to SAM addition. Both MLL1 and $WRAD_2$ were 1 µM. **A.** Substrates were 0.05 mg/mL chicken core histones, 1 µM [^{3}H]-SAM. **B.** Substrates were 50 µM H3(1-21) peptide and 10 µM [^{3}H]-SAM.

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