# Data Sheet

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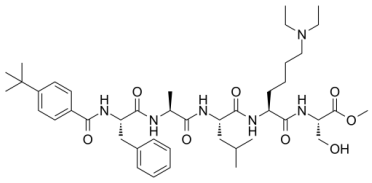
| **Product Name:** | UNC3866 |
| **Cat. No.:** | CS-6101 |
| **CAS No.:** | 1872382-47-2 |
| **Molecular Formula:** | C43H66N6O8 |
| **Molecular Weight:** | 795.02 |
| **Target:** | Histone Methyltransferase |
| **Pathway:** | Epigenetics |
| **Solubility:** | DMSO : ≥ 27 mg/mL (33.96 mM) |



## BIOLOGICAL ACTIVITY:

UNC3866 is a potent antagonist of the **CBX7**-H3 interaction as determined by AlphaScreen ($IC_{50}$=66±1.2 nM) and is more than 100-fold selective for CBX7 over the other nine members of this methyl-lysine (Kme) reader panel. IC50 & Target: IC50: 66±1.2 nM (CBX7) [1] **In Vitro:** UNC3866, a potent antagonist of the methyl-lysine (Kme) reading function of the Polycomb CBX and CDY families of chromodomains. UNC3866 binds the chromodomains of CBX4 and CBX7 most potently with a $K_d$ of 100 nM for each, and is 6- to 18-fold selective versus seven other CBX and CDY chromodomains while being highly selective versus >250 other protein targets. UNC3866 inhibits PC3 cell proliferation, a known CBX7 phenotype, while UNC4219, a methylated negative control compound, has negligible effects. UNC3866 is a potent and cellularly active antagonist of PRC1 chromodomains. UNC3866 is the most potent ligand reported for CBX7 with a $K_d$ of 97±2.4 nM. UNC3866 is equipotent for CBX4, which is most similar to CBX7, while it is 18-, 6- and 12-fold selective for CBX4/7 over CBX2, -6 and -8, respectively. Additionally, UNC3866 is 65-fold selective for CBX4/7 over CDY1 and 9-fold selective for CBX4/7 over CDYL1b and CDYL2[1].

## PROTOCOL (Extracted from published papers and Only for reference)

**Kinase Assay:** [1]The effect of UNC3866 on the methyltransferase activity of G9a, EHMT1, SUV39H1, SUV39H2, SETDB1, SETD8, SUV420H1, SUV420H2, SETD7, MLL1 trimeric complex, MLL3 tetrameric complex, EZH2 trimeric complex, PRMT1, PRMT3, PRMT5-MEP50 complex, PRMT6, PRMT7, PRMT8, PRDM9, SETD2, SMYD2, SMYD3, BCDIN3D and DNMT1 is assessed by monitoring the incorporation of tritium-labeled methyl group to lysine or arginine residues of peptide substrates using Scintillation Proximity Assay (SPA). Assays are performed in a 20 µL reaction mixture containing $^3$H-SAM at substrate concentrations close to the $K_m$ values for each enzyme. Three concentrations (1 µM, 10 µM, and 50 µM) of UNC3866 are used in all selectivity assays. To stop the enzymatic reactions, 7.5 M Guanidine hydrochloride is added, followed by 180 µL of buffer (20 mM Tris, pH 8.0). The reactions are mixed and then transferred to a 96-well FlashPlate. The reaction mixtures in Flash plates are incubated for 1 hour and the CPM are measured using a TopCount plate reader. The CPM counts in the absence of compound for each data set are defined as 100% activity. In the absence of the enzyme, the CPM counts in each data set are defined as background (0%)[1]. **Cell Assay:** UNC3866 is dissolved in DMSO and stored, and then diluted with appropriate medium before use[1]. [1]PC3 cells are seeded at 200 cells/well into 24-well plates. Cells are allowed to adhere overnight. The media (DMEM supplemented with 10 % FBS) is then exchanged with fresh media containing DMSO, UNC3866 or UNC4219. On day three, the media are exchanged with fresh media containing DMSO, UNC3866 or UNC4219. For dose-response studies, the $EC_{50}$ is derived from a six-point titration ranging from 100 µM to 0.4 µM of UNC3866 or UNC4219. At day 0, 3 or 6, cells are fixed with ice-cold methanol for 30 sec. and rehydrated with PBS. Nuclei of the cells are stained with DAPI (0.05 µg/mL) and numerated using High Content Microscopy. For dose-response studies, the cell count of UNC3866- or UNC4219-treated cells is normalized to the average cell count of DMSO-treated cells. The $EC_{50}$ is calculated using the "log[inhibitor] vs. the normalized response-Variable slope" equation in GraphPad Prism 5[1].

## References:

[1]. Stuckey JI, et al. A cellular chemical probe targeting the chromodomains of Polycomb repressive complex 1. Nat Chem Biol. 2016 Mar;12(3):180-7.

## CAIndexNames:

L-Serine, N-[4-(1,1-dimethylethyl)benzoyl]-L-phenylalanyl-L-alanyl-L-leucyl-N6,N6-diethyl-L-lysyl-, methyl ester

## SMILES:

CC(C)(C)C1=CC=C(C(N[C@@H](CC2=CC=CC=C2)C(N[C@@H](C)C(N[C@@H](CC(C)C)C(N[C@@H](CCCCN(CC)CC)C(N[C@H](C(OC)=O)CO)=O)=O)=O)=O)C=C1



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