

dCypher® K-AcylStat Nucleosome Panel

Catalog No. 16-9003
 Lot No. 19070001
 Pack Size 96 well plate



EpiCypher®

Product Description:

A 96-well plate containing 23 unique mononucleosomes assembled from recombinant human histones expressed in *E. coli* (two each of histones H2A, H2B, H3 and H4; accession numbers: H2A-P04908, H2B-O60814, H3.1-P68431/H3.2-Q71D13, H4-P62805). Single and combinatorial histone post-translational modifications (PTMs) are created using a proprietary synthetic method. The nucleosomes are wrapped by 147 base pairs DNA containing a central 601-positioning sequence (Lowary and Widom 1998 J Mol Biol. (1):19-42) with a 5' biotin-TEG group.

All nucleosomes in the panel are subjected to EpiCypher's rigorous quality control metrics, including: ESI-TOF mass spectrometry analysis of the modified proteins; SDS-PAGE to confirm octamer composition and purity; native PAGE to confirm nucleosome assembly and lack of free DNA; and Western blot analysis of the PTM. For the full list of nucleosomes in the panel, including individual catalog numbers of full-size (50 µg) products, see the associated excel sheet on the product page at www.epicypher.com.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Unmodified	H3K18cr	H4K5ac		Unmodified	H3K18cr	H4K5ac		Unmodified	H3K18cr	H4K5ac	
B	H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac		H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac		H3K4ac	tetraAc-H3 (K4/9/14/18ac)	H4K8ac	
C	H3K9ac	H3K23ac	H4K12		H3K9ac	H3K23ac	H4K12		H3K9ac	H3K23ac	H4K12	
D	H3K9bu	H3K27ac	H4K16ac		H3K9bu	H3K27ac	H4K16ac		H3K9bu	H3K27ac	H4K16ac	
E	H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)		H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)		H3K9cr	H3K27bu	tetraAc-H4 (K5/8/12/16ac)	
F	H3K14ac	H3K27cr	H4K20ac		H3K14ac	H3K27cr	H4K20ac		H3K14ac	H3K27cr	H4K20ac	
G	H3K18ac	H3K27ac + S28ph	tetraAc-H2A (K5/8/13/15ac)		H3K18ac	H3K27ac + S28ph	tetraAc-H2A (K5/8/13/15ac)		H3K18ac	H3K27ac + S28ph	tetraAc-H2A (K5/8/13/15ac)	
H	H3K18bu	H3K36ac			H3K18bu	H3K36ac			H3K18bu	H3K36ac		
	Replicate #1			Blank	Replicate #2			Blank	Replicate #3			Blank

dCypher® K-AcylStat Nucleosome Panel plate layout. The K-AcylStat panel includes an unmodified nucleosome control and 22 designer nucleosomes (dNucs: single and combinatorial PTMs), either acetylated (ac), butyrylated (bu), or crotonylated (cr) at the indicated lysine sites.

Formulation:

Purified recombinant mononucleosomes individually stored in a 96 well plate (1.5 µg nucleosome at 1.5 µM per well) in 5 µL of nucleosome storage buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 25 mM NaCl, 2 mM DTT, & 20% glycerol). MW = ~200,000 Da

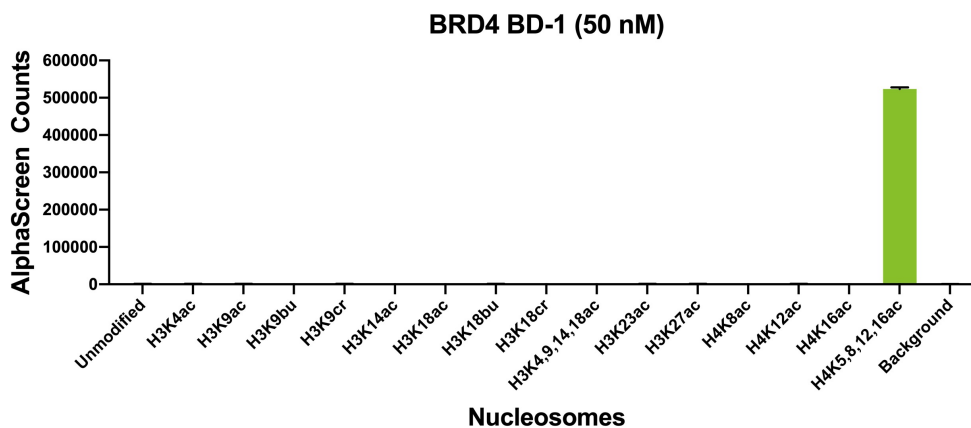
Storage and Stability:

Stable for six (6) months at -80°C from date of receipt. Avoid multiple freeze/thaws.

Application Notes:

The access to epigenetic diversity in a physiological nucleosome context enables broad end-user applications, including nucleosome binding studies (*e.g.* chromatin reader binding preferences, see application data below for example using BRD4 BD-1), enzyme screening assays (*e.g.* identification of preferred substrates), and antibody specificity testing (*e.g.* for applications that require antibody specificity in a nucleosome, rather than histone peptide context). The biotin group on the DNA facilitates pull-down experiments. EpiCypher mononucleosomes do not contain free DNA which could alter assayed activities.

This product is for *in vitro* research use only and is not intended for use in humans or animals.



Application data. GST-tagged BRD4 Bromodomain 1 (50 nM, EpiCypher Catalog No. 15-0012) was assayed in AlphaScreen (Perkin Elmer) to measure binding to nucleosomes in the dCypher® K-AcylStat Nucleosome Panel (10 nM, EpiCypher Catalog No. 16-9003). BRD4 BD-1 showed strong, specific binding to tetra-acetylated histone H4 (H4K5/8/12/16ac), consistent with its reported binding preference.

Frequently Asked Questions:

Q: How many assay points of each nucleosome are supplied?

A: This varies according to the sensitivity of the end application. For some context, the supplied format is sufficient to perform >35 AlphaScreen assays, >30 IP-qPCR experiments, and 3-5 immunoblotting experiments.

Q: How do I store the plate? Can I dilute the nucleosomes?

A: The nucleosomes are stable for 6 months at -80°C in the supplied format (1.5 µM). Freeze/thaw cycles should be limited (2-3 times). When diluted below 1.5 µM, a carrier protein (*e.g.* BSA) must be used to supplement the concentration, otherwise, stability is affected. For example, in our SNAP-ChIP product line, nucleosomes are diluted to 0.6 nM in 10 mM sodium cacodylate, pH 7.5, 100 mM NaCl, 1 mM EDTA, 50% glycerol (w/v), 1x Protease Inhibitor cocktail, 100 µg/mL BSA, 10 mM β-mercaptoethanol. In this formulation, the nucleosomes are stable at -20°C (where they do not freeze) for 6 months.

For applications that may not be compatible with the SNAP-ChIP buffer, EpiCypher routinely dilutes nucleosomes to 40 nM in assay buffer (50 mM Tris pH 7.5 + 0.01% Tween-20 + 0.01% BSA). Under these conditions the nucleosomes are stable for ~2 weeks at -20°C. *Note: extremely high amounts of salt (>650 mM) and ionic detergents (*e.g.* SDS) will disrupt nucleosome stability.

Q: How can I use a few nucleosomes in the panel and avoid freeze/thaw of the rest?

A: The nucleosomes are conveniently packaged in 8-well strip tubes and caps, so each column can be conveniently separated from the rest of the plate. For example, if the user desires to use only one of the three replicates in the plate, the tubes in columns 1, 2, and 3 can be removed without thawing the remainder of the plate.

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