Antibody: H3K9me3

Vendor: Abcam Immunogen:

Product Number: ab8898

Lot Number: 877391

Epitope: H3K9me3

Vendor: Abcam

Cat #: ab8898

lot #: 877391

Broad Institute Encode3

Antibody: H3K9me3

Vendor: Abcam

Product Number: ab8898

Lot Number: 877391

Test Date 4/21/2010 User MC

Primary Antibody H3K9me3

 Vendor
 Abcam

 Part #
 ab8898

 Lot #
 877391

 Concentration
 1 :2500

 Incubation Time
 60m

Secondary Antibody

 Vendor
 USB #72560

 Part #
 72560

 Lot #
 123941

 Concentration
 1 :10,000

 Incubation Time
 30m

Develop Time 5m



Gel Setup

	1	2	3	4	5	6
Α	K4m1	K4m2	K4m3	K9m1	K9m1	Hela
	0.01ug	0.01ug	0.01ug	0.01ug	0.1ug	0.01ug
В	K4m1	K4m2	K4m3	K9m2	K9m2	Hela
	0.1ug	0.1ug	0.1ug	0.01ug	0.1ug	0.1ug
С	K36m1	K36m2	K36m3	K9m3	K9m3	H3
	0.01ug	0.01ug	0.01ug	0.01ug	0.1ug	0.01ug
D	K36m1	K36m2	K36m3	K9ace	K9ace	H3
	0.1ug	0.1ug	0.1ug	0.01ug	0.1ug	0.1ug
Ε	K79m1	K79m2	K79m3	K27m1	K27m1	H4
	0.01ug	0.01ug	0.01ug	0.01ug	0.1ug	0.01ug
F	K79m1	K79m2	K79m3	K27m2	K27m2	H4
	0.1ug	0.1ug	0.1ug	0.01ug	0.1ug	0.1ug
G	K20m1	K20m2	K20m3	K27m3	K27m3	TBS
	0.01ug	0.01ug	0.01ug	0.01ug	0.1ug	
Н	K20m1	K20m2	K20m3	K27ace	K27ace	TBS
	0.1ug	0.1ug	0.1ug	0.01ug	0.1ug	

Protocol

- Add each peptide into BioDot at above concentrations in 50uL total volume
- Let sit on rocker 60 minutes
- Pull through with vacuum, wash once with 100uL TBS and twice with 200uL TBSTw
- Remove membrane from BioDot and transfer to 10mL Pierce SuperBlock in tray
- Place on rocker for 30 minutes
- Dump off SuperBlock and add Primary Antibody in 10mL total volume
- Place on rocker for 30 minutes
- Wash 3 x 5 minutes in TBSTw
- Add secondary antibody diluted in SuperBlock in 10mL total volume
- Place on rocker for 30 minutes
- Wash 3 x 15 minutes in TBSTw, 1 x 10 minutes in TBS
- · Develop in Fluorchem

