Histone H3 phospho Ser10 ELISA

Catalog No. 53111

Histone H3 phospho Ser28 ELISA

Catalog No. 53100

(version A)

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Overview

The addition or removal of modifications such as phospho-, methyl- and acetyl- functional groups to histones can have a profound effect on nuclear signaling as these dynamic modifications are critical in the regulation of transcription, chromosome packaging, DNA damage repair and functional genomics. Screening extracts for specific histone modifications is a simple way to assess cell health and the effect of treatment compounds on cell division.

Phosphorylation of serine 10 and serine 28 in the tail of histone H3 occur early in mitosis and serve as excellent mitotic markers. Histone H3 is phosphorylated on serine 10 during late S phase or G2 phase, while the phosphorylation of serine 28 occurs during prophase. In contrast to serine 10, serine 28 phosphorylation has never been observed in interphase.

The Histone H3 phospho Ser10 ELISA is a simple solution for screening purified core histones or extracts for levels of phosphorylated serine 10 on histone H3 in human and other (wide range predicted) systems. These kits are sandwich ELISAs that utilize a C-terminal Histone H3 antibody to capture histone H3 from your sample and a mouse monoclonal antibody phosphorylated at serine 10 on histone H3 for specific detection. A secondary antibody conjugated to horseradish peroxidase (HRP) and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry on a standard plate reader. The assay is performed in a convenient 96-stripwell plate, enabling you to simultaneously screen from 1 to 96 samples in a single experiment. It works with acid extracts from tissue or cell samples and is able to detect phospho-histone H3 (Ser10) in as little as 156 nanograms of acid extract. For added convenience, negative and positive control acid extract is also included.

The Histone H3 phospho Ser28 ELISA is a simple solution for screening purified core histones or extracts for levels of phosphorylated serine 28 on histone H3 in human, mouse, hamster and bovine systems. These kits are sandwich ELISAs that utilize a C-terminal Histone H3 antibody to capture histone H3 from your sample and a rat monoclonal antibody phosphorylated at serine 28 on histone H3 for specific detection. A secondary antibody conjugated to horseradish peroxidase (HRP) and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry on a standard plate reader. The assay is performed in a convenient 96-stripwell plate, enabling you to simultaneously screen from 1 to 96 samples in a single experiment. It works with acid extracts from tissue or cell samples and is able to detect phospho-histone H3 (Ser28) in as little as 156 nanograms of acid extract. For added convenience, negative and positive control acid extract is also included.

product	format	catalog no.
Histone H3 phospho Ser10 ELISA	1 x 96 rxns	53111
Histone H3 phospho Ser28 ELISA	1 x 96 rxns	53100

Introduction

Histone H3 phospho Serine 10 and Serine 28

The basic structural unit of chromatin is the nucleosome, which consists of 146 base pairs (bp) of DNA wrapped around a histone octamer. The histone octamer consists of two copies each of the core histone H2A-H2B dimers and a tetramer of H3-H4. A linker histone, histone H1, binds chromatin outside the nucleosome unit to regulate chromatin structure.

Histone modifications such as phosphorylation, acetylation, and methylation at specific amino acid residues on the histone tails that extend beyond the core nucleosome have been found to influence and regulate transcription, chromosome packaging, and DNA damage repair. Many of these specific histone modifications are conserved throughout eukaryotes. While the biological significance of some histone modifications remains to be understood, some have been demonstrated to correlate very closely with specific cellular states like mitosis.

Cell division is a complex, tightly regulated process that is marked by mitosis. Two significant mitotic events include microtubule formation and chromosome condensation. Phosphorylation of serine 10 and serine 28 in the tail of histone H3 occur early in mitosis when chromosomes begin to condense and during premature chromosome condensation induced in S-phase cells. Histone H3 is phosphorylated on serine 10 during late S phase or G2 phase, while the phosphorylation of serine 28 occurs during prophase[1,2]. In contrast to serine 10, serine 28 phosphorylation has never been observed in interphase[1]. Both phosphorylations occur during mitotic chromatin condensation before nuclear division occurs, which make phosphorylation of histone H3 at Ser10 or Ser28 important markers for cells undergoing mitosis.

Phosphorylated serine 28 on histone H3 has recently been shown to associate with destabilized nucleosomes in transcribed chromatin, making this an interesting indicator of both mitotic activity and transcriptional activation[3].

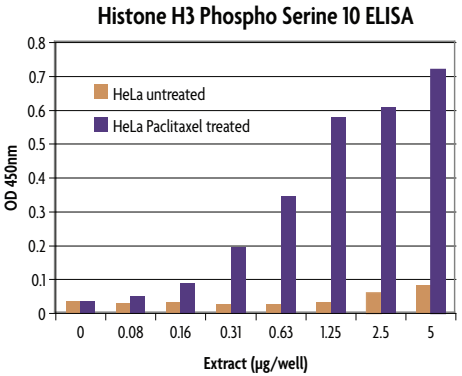
Kit Performance and Benefits

Detection limit: < 5 µg acid extract/well.

Range of detection: Histone phospho Ser10 and Ser28 ELISAs provide quantitative results from 156 ng to 5 µg of histones isolated by acid extraction.

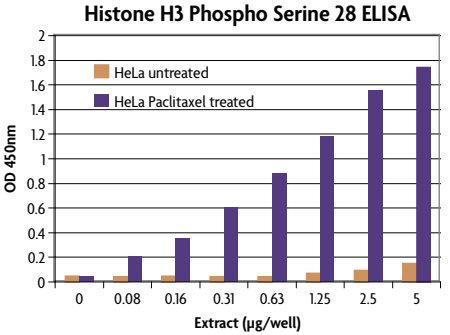
Cross-reactivity: Histone H3 phospho Ser10 Kits work with human and a wider range of species reactivity is predicted due to the high degree of sequence homology of histone H3. Histone H3 phospho Ser28 Kits work with human, mouse, hamster and bovine systems.

Assay time: 3.5 hours.



Histone H3 phospho Ser 10 levels in paclitaxel and untreated HeLa acid extracts.

78 ng to 5 µg of Paclitaxel treated (Catalog No. 36201) and untreated HeLa acid extract (Catalog No. 36200), prepared as stated in Appendix A, were assayed in the Histone H3 phospho Ser10 ELISA. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.



Histone H3 phospho Ser 28 levels in paclitaxel and untreated HeLa acid extracts.

78 ng to 5 µg of Paclitaxel treated (Catalog No. 36201) and untreated HeLa acid extract (Catalog No. 36200), prepared as stated in Appendix A, were assayed in the Histone H3 phospho Ser28 ELISA. Data shown are the results from wells assayed in duplicate. These results are provided for demonstration only.

Kit Components and Storage

Histone H3 phospho Ser10 Kits and Histone H3 phospho Ser28 Kits are for research use only. Not for use in diagnostic procedures. All components are guaranteed stable for 6 months from date of receipt when stored properly.

Reagents	Quantity	Storage
Histone H3 phospho Ser10 Mouse mAb, or Anti-phospho Histone H3 Ser28 Rat mAb	10 µl 11 µl	4°C 4°C
HRP-conjugated anti-mouse IgG, or HRP-conjugated anti-rat IgG	10 µl 10 µl	4°C 4°C
Histone H3 Capture Plate	1	4°C
Plate sealer	1	4°C
Assay Dilution Buffer AM2	15 ml	4°C
20X Wash Buffer	25 ml	4°C
Developing Solution	10 ml	4°C
Stop Solution	10 ml	4°C
Positive control HeLa acid extract (Paclitaxel treated)	25 µg/50 µl	-80°C
Negative control HeLa acid extract	25 µg/50 µl	-80°C

Additional materials required

- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Rocking platform
- Acid extracted samples or purified core histones
- Microplate spectrophotometer capable of reading at 450 nm (655 nm as optional reference wavelength)

Buffer Preparation and Recommendations

Assay Dilution Buffer AM2

Assay Dilution Buffer AM2 is provided as a 1X solution and is ready for use once thawed.

20X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 100 ml of 1X Wash Buffer required, dilute 5 ml 20X Wash Buffer with 95 ml distilled water (see Quick Chart for preparing buffers in this section). Mix gently to avoid foaming. The 1X Wash Buffer may be stored at 4°C for one week. The Tween 20 contained in the 20X Wash Buffer may form clumps, therefore homogenize the buffer by incubating at 50°C for 2 minutes and mixing prior to use.

Preparation of antibodies (see Quick Chart for preparing buffers in this section)

Dilute the Histone H3 phospho Ser 10 mouse monoclonal antibody 1:2000 with Assay Dilution Buffer AM2. Use 50 µl per well. Dilute the anti-mouse HRP-conjugated secondary 1:5000 with Assay Dilution Buffer AM2. Use 50 µl per well.

Dilute the Histone H3 phospho Ser28 rat monoclonal antibody 1:500 with Assay Dilution Buffer AM2. Use 50 µl per well. Dilute the anti-rat HRP-conjugated secondary 1:1000 with Assay Dilution Buffer AM2. Use 50 µl per well.

Developing Solution

100 µl are needed per well. The Developing Solution should be warmed to room temperature before use. The Developing Solution is light sensitive, therefore, we recommend avoiding direct exposure to intense light during storage. The Developing Solution may develop a yellow hue over time. This does not affect product performance. A blue color present in the Developing Solution indicates that it has been contaminated and must be discarded. Prior to use, place the Developing Solution at room temperature for at least 1 hour. Transfer the amount of Developing Solution required for the assay into a secondary container before aliquoting into the wells (see Quick Chart for preparing buffers in this section). After use, discard remaining Developing Solution.

Stop Solution

100 µl are needed per well. Prior to use, transfer the amount of Stop Solution required for the assay into a secondary container (see Quick Chart for preparing buffers in this section). After use, discard remaining Stop Solution.

WARNING: The Stop Solution is corrosive. Wear personal protective equipment when handling, *i.e.* safety glasses, gloves and labcoat.

Diluting HeLa acid extracts

The HeLa acid extracts (paclitaxel treated and untreated) are both provided as controls for comparing levels of phosphorylated Serine 10 or Serine 28 on Histone H3. Sufficient extract is supplied for 1 control curve of each. The paclitaxel treated HeLa acid extract is optimized to give a strong signal when used at 156 ng to 5 µg/well. The untreated HeLa acid extract is provided as a negative control. Avoid multiple freeze/thaw cycles of the extract.

Preparation of sample extracts

Histones isolated by acid extraction of tissue or cell samples or purified core histones can be used in the assay. Histone phospho Ser10 and Ser28 ELISAs provide quantitative results from 156 ng to 5 µg. It is recommended initially to use a range of sample concentrations to determine the amount of sample necessary to fall within the linear range of the assay. Once the appropriate amount of sample has been determined, perform the rest of the assays within the linear range. To prepare histones isolated by acid extraction, follow the protocol in Appendix Section A.

Quick Chart for Preparing Buffers

Reagents to prepare	Components	For 1 well	For 1 strip (8 wells)	For 6 strips (48 wells)	For 12 strips (96 wells)
Primary Antibody	Histone H3 phospho Ser10 Ab	0.026 µl	0.23 µl	1.35 µl	2.7 µl
	Assay Dilution Buffer	52 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
OR					
	Histone H3 phospho Ser28 Ab	0.1 µl	0.9 µl	5.4 µl	10.8 µl
	Assay Dilution Buffer	51.9 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
Secondary Antibody	HRP-conjugated anti-mouse IgG	0.01 µl	0.09 µl	0.54 µl	1.1 µl
	Assay Dilution Buffer	52 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
OR					
	HRP-conjugated anti-rat IgG	0.05 µl	0.45 µl	2.7 µl	5.4 µl
	Assay Dilution Buffer	52 µl	450 µl	2.7 ml	5.4 ml
	TOTAL REQUIRED	**52 µl**	**450 µl**	**2.7 ml**	**5.4 ml**
1X Wash Buffer	Distilled water	1.9 ml	17.1 ml	95 ml	190 ml
	20X Wash Buffer	100 µl	0.9 ml	5 ml	10 ml
	TOTAL REQUIRED	**2 ml**	**18 ml**	**100 ml**	**200 ml**
Developing Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**
Stop Solution	**TOTAL REQUIRED**	**112.5 µl**	**900 µl**	**5.4 ml**	**10.8 ml**

ELISA Protocol

Determine the appropriate number of microwell strips required for testing samples, controls and blanks in duplicate. If less than 8 wells in a strip need to be used, cover the unused wells with a portion of the plate sealer while you perform the assay. The content of these wells is stable at room temperature if kept dry and, therefore, can be used later for a separate assay. Store the unused strips in the aluminum pouch at 4°C. Use the strip holder for the assay.

Prepare the 1X Wash Buffer as described above in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Wash Buffer, Assay Dilution Buffer, Developing Solution and Stop Solution into the wells being used.

Control Curve Preparation (Optional)

If a control curve is not desired, skip this portion and proceed with Step 1 below.

	Untreated		Paclitaxel treated									
	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µg	5 µg	5 µg	5 µg	–	–	–	–	–	–	–	–
B	2.5 µg	2.5 µg	2.5 µg	2.5 µg	–	–	–	–	–	–	–	–
C	1.25 µg	1.25 µg	1.25 µg	1.25 µg	–	–	–	–	–	–	–	–
D	0.625 µg	0.625 µg	0.625 µg	0.625 µg	–	–	–	–	–	–	–	–
E	312 ng	312 ng	312 ng	312 ng	–	–	–	–	–	–	–	–
F	156 ng	156 ng	156 ng	156 ng	–	–	–	–	–	–	–	–
G	78 ng	78 ng	78 ng	78 ng	–	–	–	–	–	–	–	–
H	0 µg	0 µg	0 µg	0 µg	–	–	–	–	–	–	–	–

1. Dilute the untreated and paclitaxel treated HeLa acid extracts to a starting concentration of 0.1 µg/µl. The control extracts provided with the kit contain 25 µg/50 µl (0.5 µg/µl) per vial. Using the reported concentration, add 200 µl Assay Dilution Buffer AM2 to the vial and pipette up and down to mix thoroughly. The result will be 25 µg HeLa acid extract in a total volume of 250 µl = 0.1 µg/µl.
2. Add 100 µl of the diluted untreated HeLa extracts to wells A1 and A2.
3. Add 100 µl of the diluted paclitaxel treated HeLa extracts to wells A3 and A4.
4. Add 50 µl of Assay Dilution Buffer to wells B1 through H4.
5. Perform a serial two-fold dilution of the extracts by transferring 50 µl of the extracts in row A to the wells in row B.

6. Mix the contents of row B by pipetting up and down 3-5 times. Do not change pipette tips between well transfers.
7. Transfer 50 µl of the contents of row B to row C and mix, as previous.
8. Continue this process until row G is reached.
9. When row G is reached, discard 50 µl of the well contents so that the final volume is 50 µl.
10. Row H will serve as the blank wells and receives no acid extracts.

Step 1: Binding of H3 to the Capture Plate

1. In duplicate, prepare the amount of desired sample. It is recommended to try a range of sample concentrations in order to determine the amount of sample necessary for optimal detection. Add desired amount of sample in 50 µl volume to plate.
2. Incubate plate containing the optional control curve and samples for 1 hour at room temperature with mild agitation (100 rpm on a rocking platform).
3. After the incubation, wash the wells 3 times with 200 µl of Wash Buffer.

Step 2: Binding of Primary Antibody

4. **Histone H3 phospho Ser10:** Dilute the Histone H3 phospho Ser10 antibody 1:2000 in Assay Dilution Buffer AM2 and mix thoroughly.
   Histone H3 phospho Ser28: Dilute the anti-phospho-Histone H3 Ser28 antibody 1:500 in Assay Dilution Buffer AM2 and mix thoroughly.
5. Add 50 µl of diluted primary antibody to each well.
6. Incubate at room temperature for 1 hour with agitation.
7. After the incubation, wash the wells 3 times with 200 µl of Wash Buffer.

Step 3: Binding of Secondary Antibody

8. **Histone H3 phospho Ser10:** Dilute the HRP-conj. anti-mouse IgG 1:5000 in Assay Dilution Buffer AM2 and mix thoroughly.
   Histone H3 phospho Ser28: Dilute the HRP-conj. anti-rat IgG 1:1000 in Assay Dilution Buffer AM2 and mix thoroughly.
9. Add 50 µl of the diluted secondary antibody solution to each well.
10. Incubate at room temperature for 1 hour with agitation.
11. After the incubation, wash the wells 3 times with 200 µl of wash buffer.

Step 4: Colorimetric Reaction

12. Remove as much of the final wash as possible by blotting the plate on paper towels.
13. Add 100 µl of room temperature Developing Solution to each well.
14. Incubate under low light conditions for several minutes while monitoring color development. Please read the Certificate of Analysis supplied with this kit for optimal development time associated with this lot number. Monitor the blue color development until the samples turn medium to dark blue. For the optional control curve, monitor the wells containing 5 µg paclitaxel treated HeLa acid extract until they turn medium to dark blue.
15. Add 100 µl of Stop Solution to all the wells. In the presence of the acid, the blue color turns yellow.
16. Read absorbance on a spectrophotometer within 5 minutes at 450nm with an optional reference wavelength of 655 nm. Blank the plate reader according to the manufacturer's instructions using the blank wells.

    Reading the reference wavelength is optional. Most microtiter plate readers are equipped to perform dual wavelength analysis and with the appropriate software, will automatically subtract the reference wavelength absorbance from the test wavelength absorbance. If your plate reader does not have this capability, you may read the plate twice, once at 450 nm and once at 655 nm then manually subtract the 655 nm OD from the 450 nm OD values.

References

1. Goto, H., *et al.* (1999) *J. Biol. Chem.* 274(36): 25543-25549
2. Hooser, A. V. *et al.* (1998) *J. of Cell Science* 111: 3497-3506
3. Sun, J-M., *et al* (2007) *Nucleic Acids Res.* 35(19): 6640-6647

Appendix

Section A. Preparation of Acid Extract/Crude histone proteins

This procedure can be used for a 150 mm plate that is 70% confluent. The yield is approximately 0.15 mg of nuclear proteins from 9 x 10^6 cells.

1. Grow HeLa cells to 70% confluency in DMEM with 10% FBS.
2. Treat cells as desired.
3. Wash cells with 1X PBS and aspirate.
4. Add 3-5 ml of PBS per 150 mm plate.
5. Scrape cells from the plate and transfer to a 50 ml conical tube.
6. Pellet the cells by centrifugation in a pre-cooled 4°C rotor at 200 x g for 5-10 minutes.
7. Aspirate as much of the PBS as possible without disturbing the cell pellet.
8. Resuspend the cell pellet in 5 volumes of Lysis Buffer (see below).
9. Incubate on ice for 30 minutes and occasionally invert the tube to mix.
10. Centrifuge the lysate at 11,000 x *g* for 10 minutes at 4°C.
11. Collect the supernatant fraction containing acid soluble proteins, and discard the acid-insoluble pellet.
12. Immediately neutralize the acid extracted proteins by adding 2/5 the total volume of Neutralization Buffer (see below).
13. Quantify the protein concentration of your acid extraction using either gel electrophoresis or a Bradford Assay.

    Gel electrophoresis is a more sensitive technique to determine histone concentration as histones are most effectively stained by Coomassie dye in a gel matrix. To determine the protein concentration run a BSA or histone standard curve on the gel.

    A Bradford Assay can be used to determine total protein concentration, not just the concentration of crude histone proteins. A total protein determination, however, is sufficient for use in the Histone Phospho Serine ELISA Kits. The quantity of acid extract tested in the Histone Phospho Serine ELISA Kits are based on total protein determination values.
14. Immediately aliquot the extract in small volumes to avoid multiple freeze/thaws.
15. Store the protein at -80°C for long-term stability.

Lysis Buffer:

0.4 M HCl

Neutralization Buffer:

1 M Sodium Phosphate, dibasic, pH 12.5. Use 5M NaOH to adjust pH.

2.5 mM DTT

10 mM PMSF

Note: DTT and PMSF must be added immediately prior to use.

Section B: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in all wells in the correct order
	Substrate or conjugate is no longer active	Test conjugate and substrate for activity by mixing a small aliquot of HRP and Developing Solution together
	Enzyme inhibitor present	Sodium azide will inhibit the peroxidase reaction. Follow our recommendations to prepare buffers
	Plate reader settings not optimal	Verify the wavelength and filter settings in the plate reader
	Incorrect assay temperature	Bring Developing Solution and Stop Solution to room temperature before using
	Inadequate volume of Developing Solution	Check to make sure that correct volume is delivered by pipette
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the positive wells turn medium-dark blue
	Concentration of antibodies is too high	Increase antibody dilutions
	Inadequate washing	Ensure all wells are filled with Wash Buffer and follow washing recommendations
Uneven color development	Incomplete washing of wells	Ensure all wells are filled with Wash Buffer and follow washing recommendations
	Well cross-contamination	Follow washing recommendations
High background in sample wells	Too much sample per well	Decrease amount of sample per well. For acid extracts, dilute down to 1-2 µg/well
	Concentration of antibodies is too high	Perform antibody titration to determine optimal working concentration. For Histone H3 phospho Ser10, start using 1:5000 for primary antibody and 1:5000 for the secondary antibody. For Histone H3 phospho Ser28, start using 1:1000 for primary antibody and 1:2000 for the secondary antibody. The sensitivity of the assay will be decreased
No signal or weak signal in sample wells	Not enough sample per well	For extracts, make sure you are using an acid extract by following the protocol recommended in Appendix Section A. Increase amount of acid extract to 10 µg/well
No signal or weak signal in positive control curve wells	Incomplete mixing of control extracts	Upon addition of Assay Dilution Buffer AM2 to the positive and negative control HeLa acid extract vials, pipette up and down to thoroughly mix the extract. Follow the protocol to generate an optional control curve

Section C. Related Products

Histone ELISAs	Format	Catalog No.
Histone H3 dimethyl Lys9 ELISA	1 x 96 rxns	53108
Histone H3 trimethyl Lys9 ELISA	1 x 96 rxns	53109
Histone H3 trimethyl Lys27 ELISA	1 x 96 rxns	53106
Total Histone H3 ELISA	1 x 96 rxns	53110

Recombinant Methylated Histones	Format	Catalog No.
Recombinant Histone H2A	50 µg	31251
Recombinant Histone H3 (C110A)	50 µg	31207
Recombinant Histone H3 monomethyl Lys4	50 µg	31208
Recombinant Histone H3 dimethyl Lys4	50 µg	31209
Recombinant Histone H3 trimethyl Lys4	50 µg	31210
Recombinant Histone H3 monomethyl Lys9	50 µg	31211
Recombinant Histone H3 dimethyl Lys9	50 µg	31212
Recombinant Histone H3 trimethyl Lys9	50 µg	31213
Recombinant Histone H3 monomethyl Lys27	50 µg	31214
Recombinant Histone H3 dimethyl Lys27	50 µg	31215
Recombinant Histone H3 trimethyl Lys27	50 µg	31216
Recombinant Histone H3 monomethyl Lys36	50 µg	31217
Recombinant Histone H3 dimethyl Lys36	50 µg	31218
Recombinant Histone H3 trimethyl Lys36	50 µg	31219
Recombinant Histone H3 monomethyl Lys79	50 µg	31220
Recombinant Histone H3 dimethyl Lys79	50 µg	31221
Recombinant Histone H3 trimethyl Lys79	50 µg	31222
Recombinant Histone H4	50 µg	31223
Recombinant Histone H4 monomethyl Lys20	50 µg	31224
Recombinant Histone H4 dimethyl Lys20	50 µg	31225
Recombinant Histone H4 trimethyl Lys20	50 µg	31226

Control Acid Extracts	Format	Catalog No.
HeLa acid extract	100 µg	36200
HeLa acid extract (Paclitaxel treated)	100 µg	36201
HeLa acid extract (Sodium Butyrate treated)	100 µg	36202
HeLa acid extract (Etoposide treated)	100 µg	36203
HeLa acid extract (Anacardic acid treated)	100 µg	36204

DNA Methylation	Format	Catalog No.
MethylDetector™	50 rxns	55001
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003

Histone Purification	Format	Catalog No.
Histone Purification Kit	10 rxns	40025
Histone Purification Mini Kit	20 rxns	40026

Chromatin Assembly	Format	Catalog No.
Chromatin Assembly Kit	10 rxns	53500
HeLa Core Histones	36 µg	53501

Histone Acetyltransferase and Deacetylase Activity	Format	Catalog No.
HAT Assay Kit (Fluorescent)	1 x 96 rxns	56100
Recombinant p300 protein, catalytic domain	5 µg	31205
HDAC Assay Kit (Fluorescent)	1 x 96 rxns	56200
HDAC Assay Kit (Colorimetric)	1 x 96 rxns	56210

Co-Immunoprecipitation	Format	Catalog No.
Nuclear Complex Co-IP Kit	50 rxns	54001
Universal Magnetic Co-IP Kit	25 rxns	54002

SUMOylation	Format	Catalog No.
SUMOlink™ SUMO-1 Kit	20 rxns	40120
SUMOlink™ SUMO-2/3 Kit	20 rxns	40220

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express HT	96 rxns	53018
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Re-ChIP-IT™	25 rxns	53016
ChIP-IT™	25 rxns	53001
ChIP-IT™ w/o controls	25 rxns	53004
ChIP-IT™ Shearing Kit	10 rxns	53002
ChIP-IT™ Enzymatic	25 rxns	53006
ChIP-IT™ Enzymatic w/o controls	25 rxns	53007
Enzymatic Shearing Kit	10 rxns	53005
Salmon Sperm DNA/Protein G agarose	25 rxns	53003
ChIP-IT™ Control Kit – Human	5 rxns	53010
ChIP-IT™ Control Kit – Mouse	5 rxns	53011
ChIP-IT™ Control Kit – Rat	5 rxns	53012
Ready-to-ChIP HeLa Chromatin	10 rxns	53015
Ready-to-ChIP Hep G2 Chromatin	10 rxns	53019
Ready-to-ChIP K-562 Chromatin	10 rxns	53020
Ready-to-ChIP NIH/3T3 Chromatin	10 rxns	53021

Active Motif also offers a growing list of application validated antibodies, including antibodies for histones and histone modifications, transcription factor antibodies, DNA methylation-related antibodies and ChIP validated antibodies. For a complete list go to **www.activemotif.com/abs**

Transcription Factor ELISAs	Format	Catalog No.
TransAM™ AP-1 Family	2 x 96-well plates	44296
TransAM™ AP-1 c-Jun	1 x 96-well plate	46096
TransAM™ GR	1 x 96-well plate	45496
TransAM™ HIF-1	1 x 96-well plate	47096
TransAM™ IRF-3 (Human)	1 x 96-well plate	48396
TransAM™ IRF-7	1 x 96-well plate	50196
TransAM™ NFATc1	1 x 96-well plate	40296
TransAM™ NFκB Family	2 x 96-well plates	43296
TransAM™ NFκB p50	1 x 96-well plate	41096
TransAM™ NFκB p52	1 x 96-well plate	48196
TransAM™ NFκB p65	1 x 96-well plate	40096
TransAM™ p53	1 x 96-well plate	41196
TransAM™ STAT Family	2 x 96-well plates	42296

For a complete list of the over 40 TransAM Kits available, please visit **www.activemotif.com/transam**

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If you need assistance at any time, please call Active Motif Technical Service at one of the numbers listed below.

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