GenoMatrix™ Whole Genome Amplification Kit

(version A3)

Catalog No. 58001

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TABLE OF CONTENTS **Page**

Overview

Whole Genome Amplification (WGA) has emerged as a fundamental method for DNA analysis from limited quantities of genomic DNA in forensic analysis and disease gene discovery. The WGA technique allows for amplification of the whole genome, preserving what was a limited sample while enabling analysis of any gene in the organism being studied. Active Motif's GenoMatrix™ Whole Genome Amplification Kit makes it possible for users to achieve up to a 500-fold amplification of genomic DNA starting from nanogram quantities of DNA while maintaining the sequence representation of the starting material. Several strategies for WGA have been developed during the past decade, each with variable fidelity, yield and coverage of the amplified genome (see References for examples of these different methods). The GenoMatrix Kit provides all the reagents and buffers to quickly and easily perform a genome-representative amplification of any type of genomic DNA. In particular, the method works especially well with ChIP samples as well as samples generated using Active Motif's MethylCollector™ and UnMethylCollector™ Kits. All reagents included in the GenoMatrix Whole Genome Amplification Kit have been optimized for high-efficiency genome amplification.

product	format	catalog no.
GenoMatrix™ Whole Genome Amplification Kit	50 rxns	58001

The GenoMatrix™ Whole Genome Amplification Kit is for research use only. Not for use in diagnostic procedures.

GenoMatrix Whole Genome Amplification Kit Advantages

Preserves the Sequence Representation of the Starting Genomic DNA

Active Motif's GenoMatrix Whole Genome Amplification Kit utilizes a new approach that virtually eliminates amplification bias, so the amplified material has the same sequence representation as the material that you started with.

Quick and Simple

The entire procedure can be completed in less than two and half hours.

Versatile

GenoMatrix can be used as a stand alone product to amplify unsheared DNA or with samples generated by Active Motif's ChIP-IT™ Express, MethylCollector™ and UnMethylCollector™ Kits.

Kit Components and Storage

Please store each component at the temperature indicated in the table below.

Reagents	Quantity	Storage / Stability
10X Shearing Buffer	55 µl	-20°C for 6 months
GenoMatrix Buffer 1	200 µl	-20°C for 6 months
GenoMatrix Buffer 2	250 µl	-20°C for 6 months
GenoMatrix DNA polymerase	55 µl	-20°C for 6 months
GenoMatrix Primer A	60 µl	-20°C for 6 months
GenoMatrix Primer B	150 µl	-20°C for 6 months
10X PCR Buffer	1.5 ml	-20°C for 6 months
Taq DNA polymerase	45 µl	-20°C for 6 months
dNTPs (5 mM each)	450 µl	-20°C for 6 months
Human Genomic Control DNA	12 µl	-20°C for 6 months

Additional materials required

- Sterile water
- Pipettors and filter tips
- PCR tubes
- Thermal cycler
- DNA purification columns (*e.g.* Active Motif's Chromatin IP DNA Purification Kit, Catalog No. 58002). Any columns used should have the ability to retain ssDNA as well as dsDNA.
- Spectrophotometer or equivalent to quantify DNA. The method used must be able to quantify ssDNA as well dsDNA. (Measurement techniques such as PicoGreen that do not detect ssDNA will often understate the total amount of DNA present, as single-stranded DNA may be generated during amplification.)

GenoMatrix Whole Genome Amplification Kit
Experimental Design

PLEASE READ THE ENTIRE PROTOCOL BEFORE STARTING!

Active Motif's GenoMatrix Whole Genome Amplification Kit includes sufficient reagents to perform 50 whole genome amplification reactions. Each sample must contain a minimum of 10 ng of DNA for the process to work efficiently. The kit enables you to generate a representative amplification of any kind of genomic DNA.

The GenoMatrix Whole Genome Amplification process is divided into DNA fragmentation, library preparation and library amplification. The first two steps, fragmentation and library preparation, should be carried out without interruption, as the ends of the library DNA can degrade.

The library DNA can be stored up to one week at -20°C without detectable differences in the results. The final GenoMatrix Whole Genome Amplification DNA should be stored at -20°C and is as stable as any comparable genomic DNA.

Step 1 (Protocol A)
The starting DNA samples are subjected to a random shearing.

> **Note:** This step is omitted if the DNA samples have already been sheared by another method, such as subsequent to chromatin immunoprecipitation (ChIP) or as the result of MethylCollector or UnMethylCollector experiments.

Step 2 (Protocol B)
In the second step, the sheared DNA samples are converted from small DNA fragments of unknown sequence into PCR amplifiable molecules flanked by known sequences.

Step 3 (Protocol C)
The resultant DNA from Step 2 are then PCR amplified using specific oligonucleotide primers. The kit requires a minimum of 10 ng of starting DNA, which can yield up to 5 µg of Whole Genome Amplified product (up to a 500-fold amplification of the original genomic DNA). The GenoMatrix Whole Genome Amplification Kit contains an optimized DNA polymerase that decreases the background in the reaction. After purification, the amplified product can be analyzed in a manner similar to any DNA sample. A number of downstream applications may be performed including microarray hybridizations, quantitative PCR, *etc*.

Notes Before Starting:

- Always run a control sample with water only (no DNA template) to ensure that the reagents are not contaminated with DNA.
- Run a positive control sample such as the human genomic control DNA included in the kit along with your samples.
- Use filter tips and gloves during the entire procedure to prevent DNA contamination.

Protocols

The GenoMatrix Whole Genome Amplification Kit requires a minimum of 10 ng starting DNA in a volume of no more than 11 µl. Insufficient (or too dilute) starting material can result in poor genomic representation in the final product.

It is recommended that you perform the protocol in 0.2 ml PCR tubes so that you can use a thermal cycler for the incubation steps.

If you are working with unsheared genomic DNA, begin with Protocol A. If your samples are the result of chromatin immunoprecipitation (ChIP), MethylCollector, UnMethylCollector or some other procedure that results in sheared DNA, begin with Protocol B.

See the Appendix for information regarding the use of GenoMatrix with ChIP samples (Appendix – Section A) or with MethylCollector and UnMethylCollector samples (Appendix – Section B).

A. DNA Shearing

1. Quantify your DNA sample, then prepare a 1 ng/µl DNA solution.
2. Add 10 µl of the DNA solution (1 ng/µl) to a clean PCR tube.
3. Add 1 µl of 10X Shearing Buffer the 10 µl of DNA solution in the PCR tube.
4. Place the tube in a thermal cycler and heat at 95°C for exactly 4 minutes.
5. Immediately cool the sample on ice, then centrifuge briefly to collect the liquid in the bottom of the tube. Return the sample to ice or 4°C immediately after centrifugation.

B. Preparation of PCR-amplifiable DNA Fragment Library

1. Add 1 µl of GenoMatrix Primer A (40 µM) to each sample.
2. Add 3 µl of GenoMatrix Buffer 1 and mix well by vortexing. If necessary, centrifuge briefly to collect the liquid in the bottom of the tube.
3. Heat for 2 minutes at 95°C (in a thermal cycler). Cool immediately on ice, centrifuge briefly and return to ice for 5 minutes.
4. Add 4 µl of GenoMatrix Buffer 2 to samples.
5. Add 1 µl of GenoMatrix DNA polymerase.
6. Vortex thoroughly and centrifuge briefly.

7. Place samples in a thermal cycler and incubate as follows:
   a. 16°C for 20 minutes
   b. 24°C for 20 minutes
   c. 37°C for 20 minutes
   d. 75°C for 5 minutes

   Hold at 4°C
8. Remove samples from thermal cycler and centrifuge briefly. Samples may be amplified immediately or stored at -20°C for up to one week before Library Amplification.

Section C. Library Amplification

1. Dilute samples with distilled water to a final volume of 60 µl (add 40 µl of sterile dH_2O).
2. Prepare a master mix with the following components:

Reagent	Per Reaction
10X PCR Buffer	10 µl
dNTPs	8 µl
GenoMatrix Primer B	2 µl
Taq DNA polymerase	0.8 µl
dH_2O	64.2 µl
Total	**85 µl**

In fresh PCR tubes, add 85 µl of this mix to 15 µl of your diluted sample.

3. Put samples in a thermal cycler and perform 20-30 cycles as follows:
   a. 94°C for 30 seconds
   b. 40°C for 30 seconds
   c. 50°C for 30 seconds
   d. 72°C for 2 minutes

   Hold at 4°C
4. Remove samples from thermal cycler and centrifuge briefly to collect the liquid in the bottom of the tube. Maintain the reactions at 4°C or store at -20°C until ready for analysis or purification. The GenoMatrix Whole Genome Amplification DNA is now as stable as any type of genomic DNA stored under the same conditions.

The quality and the average size of the GenoMatrix Whole Genome Amplification DNA can also be determined by loading 5-10 µl of the final product on a 1.5-2% agarose gel. A "smear" of DNA should be present between 300 bp and 1 kb.

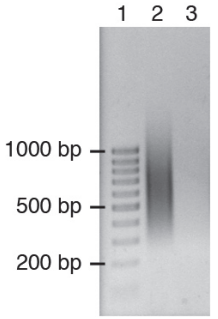

Figure 1. Agarose gel displaying the results of the GenoMatrix Whole Genome Amplification Kit.

Lane 1: DNA molecular weight marker.

Lane 2: GenoMatrix Kit using 10 ng of Human Genomic Control DNA as the starting material.

Lane 3: GenoMatrix Kit negative control with no starting DNA.

It is recommended to purify the final product before being it is used in subsequent applications (DNA microarray hybridization, *etc.*). We recommend using Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002) or equivalent columns that retain single-stranded DNA as well as double-stranded DNA. Once purified, the DNA can be quantified by measuring absorbance assuming that 1 A_{260} unit is equivalent to 50 ng/µl DNA.

References

1. Sun G. *et al.*, 2005 *Legal Med* 7: 279-286.
2. Barker D.L. *et al.*, 2004 *Genome Research*, 14: 901-907.
3. Hughes S. *et al.*, 2005 *Prog Biophys Molec Biol* 88: 173-189.
4. Lasken R.S. and Egholm M., 2003 *Trends Biotech* 21: 531-535.
5. Hawkins T.L., Detter J.C., Richardson P.M., 2002 *Curr Opin Biotech* 13: 65-67.
6. Dean F.B. *et al.*, 2002 *PNAS* 99: 5261-5266.

Appendix

Section A. Whole Genome Amplification from ChIP Samples

At the end of chromatin immunoprecipitation (ChIP), the amounts of DNA collected are often very low. Certain techniques (*e.g.* microarray hybridization) require a larger amount of DNA than results from the average ChIP experiment. You should determine the minimal amount of chromatin you must start with in order to collect at least 10 ng of DNA.

To help you, the following chart estimates the ChIP output (DNA collected) for different quantities of starting chromatin.

Number of cells	Chromatin input	ChIP DNA output
1×10^4	200 ng	30 pg^
1×10^5	2 µg	300 pg^
5×10^5	10 µg	1.3 ng*
1×10^6	20 µg	10 ng
1×10^7	200 µg	150 ng*

* output measured from a RNA pol II ChIP sample

^ output estimated based on ChIP samples from larger scale experiments

Table 1. Estimates of DNA resulting from ChIP based on the number of mammalian cells used for each ChIP.

Depending on the efficiency of the antibody being used for ChIP and on the protein that is being immunoprecipitated, the amount of DNA collected will vary. With histone antibodies, the collected amounts of DNAs are often larger. Inversely, with antibodies targeted to rare proteins, less DNA will be collected, so you should perform your ChIP beginning with more chromatin.

Before starting the GenoMatrix Whole Genome Amplification procedure, a purification of your ChIP samples is required. This will remove anything in the ChIP buffers that might prevent amplification of the DNA. It also allows you to concentrate your samples, which is important because for the GenoMatrix procedure to work effectively, the volume of your DNA samples should not exceed 10 µl. We recommend using the Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002) or equivalent. Samples should be eluted in 10 µl of distilled water. An alternative is phenol-chloroform extraction / ethanol precipitation. For maximum efficiency, include glycogen in the precipitation. After washing and drying the pellet, resuspend it with 10 µl distilled water. One microliter of purified DNA is then used for quantification.

Once your ChIP DNA samples have been purified and quantified, the GenoMatrix procedure can be started. But, as ChIP DNA has already been sheared, you do not begin with the fragmentation step. Begin with Protocol B on page 4. Make sure that your ChIP DNA is 1 ng/µl, and use 10 µl of it to prepare the PCR-amplifiable DNA Fragment Library.

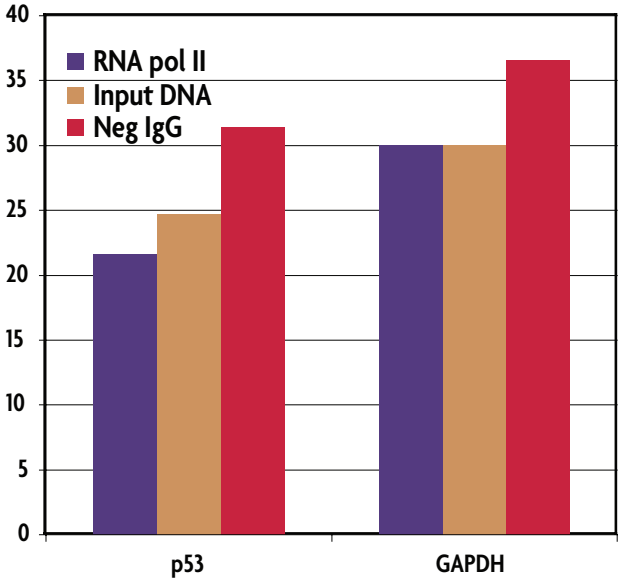

Figure 2. Quantitative PCR carried out on ChIP DNA samples

Following chromatin immunoprecipitation with RNA polymerase II antibody (purple bars) and Negative control IgG (red bars), the ChIP DNA samples were amplified using the GenoMatrix Whole Genome Amplification Kit. qPCR was then performed on the amplified samples and on the Input DNA used in the ChIP with primers specific for either the p53 or GAPDH promoters.

Section B. Whole Genome Amplification Starting with Samples Resulting from Experiments using the MethylCollector Family of DNA Methylation Detection Kits

The GenoMatrix Whole Genome Amplification procedure requires a minimum of 10 ng of purified CpG methylated or unmethylated DNA as starting material. However, if MethylCollector Ultra (Catalog No. 55005) or UnMethylCollector (Catalog No. 55004) Kits will be used to enrich for methylated or unmethylated CpG islands before beginning the GenoMatrix procedure, we recommend using 500 ng of fragmented DNA as the starting material in either the MethylCollector Ultra or UnMethylCollector Kits. The amount of DNA recovered from the MethylCollector Ultra and UnMethylCollector assays will depend on the global methylation status of the sample DNA. By starting with 500 ng of fragmented DNA, it should be possible to recover 10 ng or more after enrichment and purification of the sample.

Following enrichment, the samples must purified before starting the GenoMatrix procedure. Samples can be purified either by performing a phenol/chloroform extraction followed by ethanol precipitation, or by using Active Motif's Chromatin IP DNA Purification Kit (Catalog No. 58002). Purified DNA should be eluted in 10 µl DNase-free water. Use 1 µl of your sample for quantification. A minimum of 10 ng DNA is required before starting the GenoMatrix Whole Genome Amplification procedure.

After quantification of the purified CpG methylated or unmethylated DNA, the DNA should be diluted to a final concentration of 1 ng/µl in DNase-free water and 10 µl (10 ng) will be used in the GenoMatrix Whole Genome Amplification Kit. Because the starting material for MethylCollector Ultra and UnMethylCollector was fragmented (either by enzymatic digestion or sonication), the fragmentation step can be skipped. Begin the GenoMatrix procedure with Protocol B on page 4.

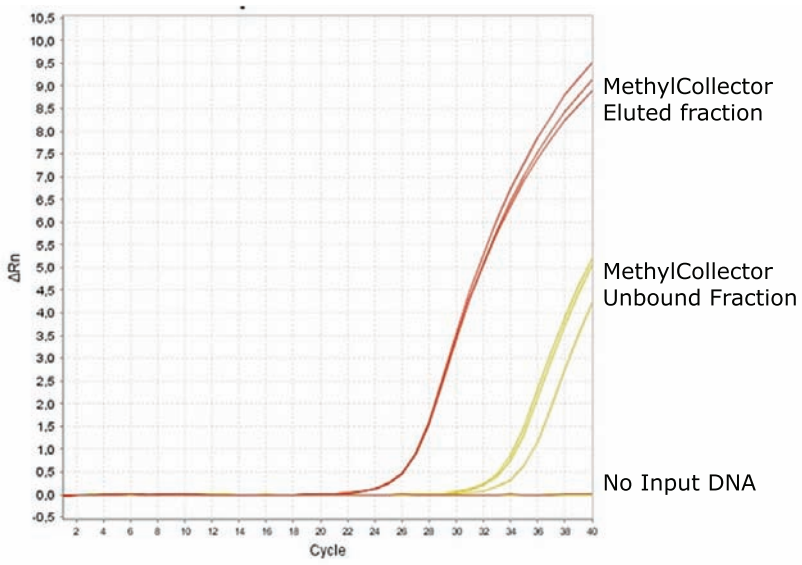

Figure 3. Real time quantitative PCR used to analyze samples isolated using MethylCollector Ultra.

500 ng of human male genomic DNA was processed using the MethylCollector Ultra Kit. Eluted and Unbound DNA samples were purified and 10 ng of each fraction was subjected to the GenoMatrix Whole Genome Amplification procedure. Then, 10 ng of purified GenoMatrix DNA samples were analyzed by real time PCR using primers specific for the promoter of a methylated gene, SNRPN, which should be bound by the MethylCollector assay, and end up in the Eluted fraction.

Section C. Representative, Comparative Data from Multiple Loci

For any Whole Genome Amplification procedure to be effective, it must to amplify the genomic DNA while preserving the DNA sequence representation of the starting material. The GenoMatrix Whole Genome Amplification Kit has been validated using primers spanning the genome, as well as used in comparison to a competitor's whole genome amplification kit.

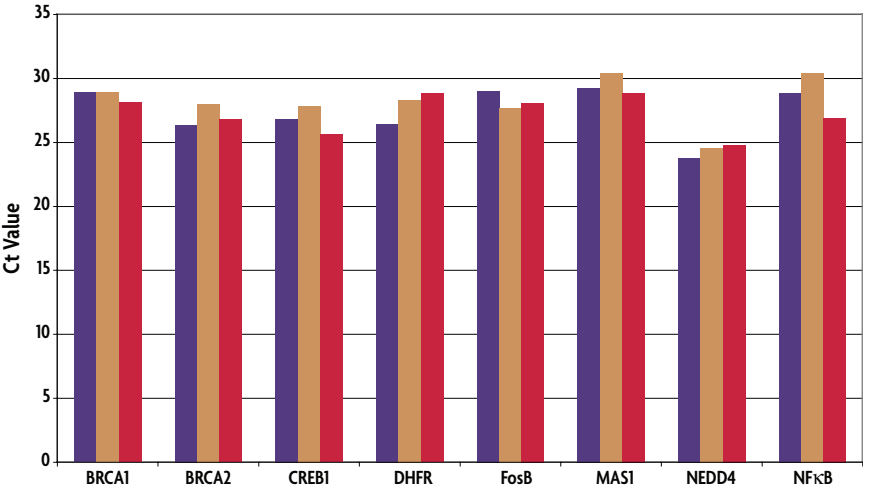

Figure 4. Quantitative PCR carried out on DNA samples using primers from a sampling of representative genes.

Quantitative real-time PCR was carried out on DNA samples using primers indicated below each data set and the Ct value for each sample plotted. The DNA was derived from an original DNA sample amplified using the GenoMatrix Whole Genome Amplification Kit (red bars), amplified with a competitor's whole genome amplification kit (copper bars) or not subjected to whole genome amplification (purple bars).

Section D. Related Products

ChIP-IT™ Kits	Format	Catalog No.
ChIP-IT™ Express	25 rxns	53008
ChIP-IT™ Express Enzymatic	25 rxns	53009
ChIP-IT™ Express Shearing Kit	10 rxns	53032
ChIP-IT™ Express Enzymatic Shearing Kit	10 rxns	53035
ChIP-IT™ Express HT	96 rxns	53018
Re-ChIP-IT™	25 rxns	53016
Chromatin IP DNA Purification Kit	50 rxns	58002
EpiShear™ Sonicator	110 V	53051
ChIP-IT™ Protein G Magnetic Beads	25 rxns	53014
Siliconized Tubes, 1.7 ml	25 tubes	53036
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
Bridging Antibody for Mouse IgG	500 µg	53017

DNA Methylation	Format	Catalog No.
hMeDIP	10 rxns	55010
MethylDetector™	50 rxns	55001
MethylCollector™	25 rxns	55002
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
DNMT Activity / Inhibition Assay	96 rxns	55006
Methylated DNA Standard Kit	3 x 2.5 µg	55008
Fully Methylated Jurkat DNA	10 µg	55003
Jurkat genomic DNA	10 µg	55007

ChIP-validated Histone Antibodies	Application	Format	Catalog No.
Histone H2A pAb	ChIP, WB	200 µl	39235
Histone H2A phospho Ser129 pAb	ChIP, IF, IP, WB	200 µl	39271
Histone H2A.Z pAb	ChIP, WB	200 µl	39113
Histone H2B pAb	ChIP, WB	200 µl	39237
Histone H2B acetyl Lys5 pAb	ChIP, WB	200 µl	39123
Histone H2B acetyl Lys12 pAb	ChIP, WB	100 µl	39669
Histone H2B acetyl Lys16 pAb	ChIP, WB	200 µl	39121
Histone H2B acetyl Lys46 pAb	ChIP, WB	200 µl	39571
Histone H2B acetyl Lys120 pAb	ChIP, WB	200 µl	39119
Histone H3, C-terminal pAb	ChIP, WB	200 µl	39163
Histone H3 acetyl pAb	ChIP, WB	200 µl	39139
Histone H3 acetyl Lys4 pAb	ChIP, IF, WB	200 µl	39381
Histone H3 monomethyl Lys4 mAb	ChIP, WB	100 µg	39635
Histone H3 dimethyl Lys4 pAb	ChIP, WB	200 µl	39141
Histone H3 trimethyl Lys4 pAb	ChIP, WB	200 µl	39159
Histone H3 dimethyl Lys9 pAb	ChIP, IF, WB	200 µl	39239
Histone H3 trimethyl Lys9 pAb	ChIP, WB	200 µl	39161
Histone H3 acetyl Lys9 pAb	ChIP, WB	200 µl	39137
Histone H3 acetyl Lys14 pAb	ChIP, WB	200 µl	39599
Histone H3 acetyl Lys18 pAb	ChIP, IF, WB	200 µl	39587
Histone H3 acetyl Lys23 pAb	ChIP, IF, WB	200 µl	39133
Histone H3 acetyl Lys27 pAb	ChIP, IF, WB	200 µg	39133
Histone H3 acetyl Lys27 pAb	ChIP, WB	200 µl	39135
Histone H3 dimethyl Lys27 pAb	ChIP, IF, WB	200 µl	39245
Histone H3 trimethyl Lys27 mAb	ChIP, WB	200 µl	39535
Histone H3 trimethyl Lys27 pAb	ChIP, IF, WB	200 µg	39155
Histone H3 trimethyl Lys27 pAb	ChIP, WB	200 µl	39156
Histone H3 acetyl Lys36 pAb	ChIP, IF, WB	200 µl	39379
Histone H3 acetyl Lys56 pAb	ChIP, WB	200 µl	39281
Histone H3 acetyl Lys64 pAb	ChIP, IF, WB	200 µl	39545
Histone H3 acetyl Lys79 pAb	ChIP, WB	200 µl	39565
Histone H4 pan-acetyl pAb	ChIP, IF, WB	200 µl	39243
Histone H4 tetra-acetyl pAb	ChIP, WB	50 µl	39179
Histone H4 acetyl Lys5 pAb	ChIP, IF, WB	200 µl	39169
Histone H4 acetyl Lys5 pAb	ChIP, IF, WB	200 µl	39583
Histone H4 acetyl Lys12 pAb	ChIP, IF, WB	200 µl	39165
Histone H4 acetyl Lys16 pAb	ChIP, WB	200 µl	39167
Histone H4 monomethyl Lys20 pAb	ChIP, IF, WB	200 µl	39175
Histone H4 trimethyl Lys20 pAb	ChIP, IF, WB	200 µl	39180

For an up-to-date list of over 125 ChIP-validated antibodies, please visit **www.activemotif.com/chipabs**.

Application Key: ChIP = Chromatin Immunoprecipitation; **EMSA** = Electrophoretic Mobility Shift Assay; **IF** = Immunofluorescence; **IHC** = Immunohistochemistry; **IP** = Immunoprecipitation; **WB** = Western blot

Technical Services

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