Nu.Q™ H3.1 Assay Kit

Powered by VolitionRx Limited

(version A3)

Catalog No. 53511

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Revision History

Revision	Date	Description of Change
A2	May 2019	Corrected Kit Components to reflect inclusion of a detection antibody that needs to be diluted 1:1000 prior to use.
A3	June 2019	Updates to Overview and Troubleshooting sections

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Overview

Nucleosomes are repeating subunits of DNA and histone proteins that constitute human chromatin. Increased levels of nucleosomes in the blood have been associated with a number of pathological conditions and likely constitutes a major proportion of the cell free DNA (cfDNA) found in the bloodstream. Thus, quantification of the levels of circulating nucleosomes in serum gives insight into the progression of many conditions.

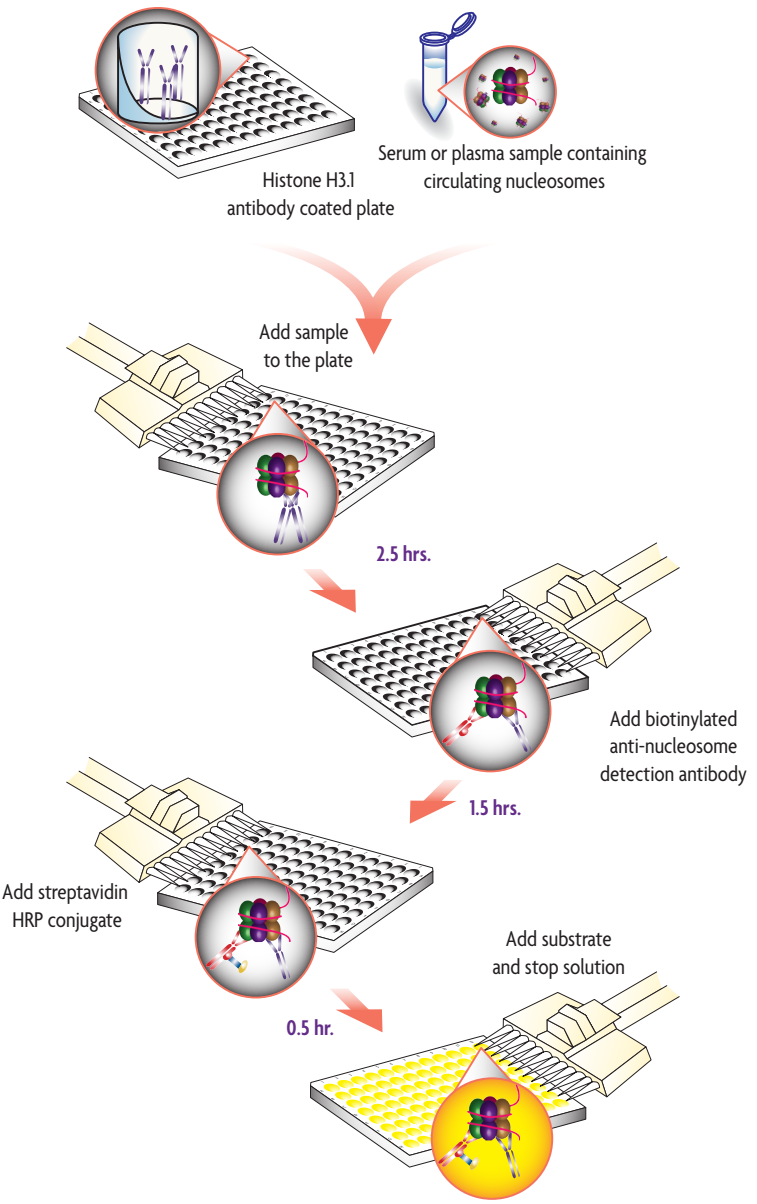
Active Motif's Nu.Q™ H3.1 Assay Kit is designed for the detection of levels of Histone H3.1 containing circulating nucleosomes in human serum and plasma in a high-throughput format to allow for a large number of samples to be screened simultaneously. This kit is a sandwich ELISA that utilizes a Histone H3.1 specific recombinant antibody to capture nucleosomes from your serum or plasma samples. Subsequently, a biotinylated recombinant antibody specific to a common nucleosome epitope is used for detection to assure that only intact nucleosomes are quanitifed in the assay. Streptavidin conjugated to horseradish peroxidase (Streptavidin-HRP conjugate) and developing solutions provide a sensitive colorimetric readout that is easily quantified by spectrophotometry. This assay is able to detect total levels of circulating nucleosomes, regardless of the modification state, from as little as 10 µl of human serum or 20 µl of human plasma.

The Nu.Q™ H3.1 Assay Kit contains enough reagents to be performed in a convenient 96-stripwell plate, enabling you to simultaneously screen up to 96 samples in a single experiment. For added convenience and a more quantitative interpretation of the results, the kit also includes the Active Motif recombinant nucleosome technology. The positive control consists of recombinant nucleosomes which enables you to build a reference standard curve to determine the total amount of circulating nucleosomes in your serum or plasma samples.

product	format	catalog no.
Nu.Q™ H3.1 Assay Kit	1 x 96 rxns	53511

The Nu.Q™ H3.1 Assay Kit is powered by VolitionRx Limited.

Flow Chart of process



Introduction

Nucleosomes comprise the smallest subunit of chromatin and consist of 147-167 bp of DNA wrapped around an octamer of core histone proteins (H2A, H2B, H3 and H4). During cellular damage, such as apoptosis or necrosis, chromatin is fragmented into oligo- or mono- nucleosomes which are released into the blood stream[1,2]. Several reports suggest that circulating nucleosomes constitute a major proportion of the cell-free DNA found in the blood[3,4], due to the DNA being protected from further plasmatic digestions[5].

Increased levels of nucleosomes in the blood have been associated with tumor burden and malignant progression in several types of cancers[6-8], as well as in degenerative diseases, autoimmune disease, ischemia, and trauma[6]. Histones and DNA are also subject to a variety of epigenetic modifications. Genome wide epigenetic signaling has been shown to be altered in cancer cells and accumulating evidence indicates that these epigenetic changes occur early in tumorogenesis[9]. The most studied potential epigenetic circulating cell-free DNA (ccfDNA) biomarkers in cancer are the methylation levels of a variety of tumor suppressor genes, particularly the septin-9 gene that show promising results in colorectal cancer detection[10-12].

Active Motif's Nu.Q™ H3.1 Assay Kit provides material for the detection of the levels of Histone H3.1 containing circulating nucleosomes in human serum or plasma and is intended for research use only. This colorimetric enzyme immunoassay (ELISA) is designed for 96-well plate format. Thus, multiple samples can be run in a single assay, in only 5 hours. Included in the kit is a recombinant Histone H3.1 nucleosome positive control and instructions for the preparation of a standard curve for validation and relative quantification of circulating nucleosome levels in the clinical samples..

References

1. Holdenrieder S. *et al.* (2001) *Clin Chem Lab Med.*, 39(7): 596-605.
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4. Chan, KC. *et al.* (2003) *Cancer Res.,* 63: 2028-2032.
5. Luger, K. *et al.* (2003) *Curr Opin Genet Dev.,* 13: 127-135.
6. Holdenrieder, S. *et al.* (2001) *Ann N Y Acad Sci.,* 945: 93-102.
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8. Ishimali, YI. *et al.* (2013) *Int J Mol Sci.,* 14(9): 18925-18958.
9. Fullgrabe, J. *et al.* (2011) *Oncogene,* 30(31): 3391-3403.
10. Summers, T. *et al.* (2013) *Oncogene,* 4(3): 210-216.
11. Jin, H. *et al.* (2013) *Rijeka: InTech.,* 137-152.
12. deVos, T. *et al.* (2009) *Clin Chem.,* 55(7): 1337-1346.

Kit Performance and Benefits

Nu.Q™ H3.1 Advantages:

Sensitivity: Detect circulating nucleosomes in as little as 10 µl of serum or 20 µl of plasma

Specificity: Nuclesosome epitope specific antibody enables detection of only intact nucleosomes

Quantification: Recombinant nucleosomes enable relative quantification of circulating H3.1 containing nucleosomes

Convenience: Colorimetric assay in a simple 96-stripwell format for high and low throughput

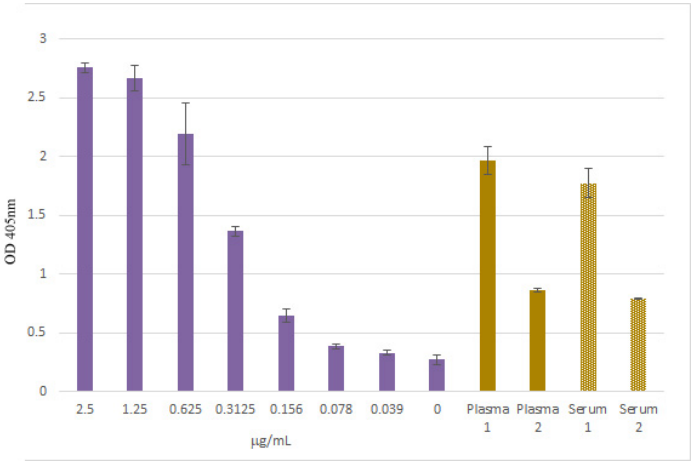
Fast: Results can be obtained in 5 hours

Detection Limit and range of detection: The detection limit of recombinant nucleosomes is as low as 0.053 µg/mL with a dynamic range of 0.078 - 2.5 µg/mL within the indicated amount of sample.

Cross-reactivity: Human, mouse, rat, yeast, and a wider range of species reactivity is predicted due to the high degree of sequence homology of nucleosome and histone H3 epitopes.

Assay time: 5 hours.

Nu.Q™ H3.1 Assay Kit



Total H3.1 circulating cell-free nucleosome detection.

The Nu.Q™ H3.1 Assay Kit was used to assay human serum and plasma samples. The provided Positive Control was assayed from 0.039 - 2.5 µg/ml as a reference standard curve. Data shown are the results from wells assayed in duplicate with a 20 minute developing time. These results are provided for demonstration only.

Kit Components & Storage

The Nu.Q™ H3.1 Assay Kit is 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
Sealing Film	3	RT
Stop Solution	10 ml	RT
Wash Buffer AM9	24 ml	4°C
Assay Buffer	9 ml	4°C
Control Diluent	1 ml	4°C
Detection Diluent	12 ml	4°C
Streptavidin-HRP conjugate	10 ml	4°C
Substrate Solution	10 ml	4°C
Nu.Q™ H3.1 Plate	1	4°C
Nu.Q™ H3.1 Detection Antibody	12 µl	-20°C
Nu.Q™ H3.1 Positive Control	10 µl	-80°C

Additional materials required

- Serum or plasma samples
- Distilled water
- Multi-channel pipettor
- Multi-channel pipettor reservoirs
- Vortex mixer
- Microplate incubator with orbital shaker (~700 rpm)
- Microplate spectrophotometer capable of reading at 405nm
- Absorbent paper

Buffer Preparation and Recommendations

Allow all buffers to warm up to room temperature for at least 30 minutes and gently homogenize each of them before use!

Nu.Q™ Assay Buffer

Is supplied ready-to-use.

Nu.Q™ Detection Antibody

Dilute the Nu.Q™ Detection Antibody 1:1000 with Nu.Q™ Detection Antibody Diluent. Use 100 µl per well (see the Quick Chart for Preparing Buffers in this section).

Nu.Q™ Positive Control

The Recombinant Histone H3.1 Nucleosome Positive Control is provided at a concentration of 75 µg/ml to allow for the quantification of circulating nucleosomes in your serum samples. There is enough recombinant nucleosomes for at least 4 and up to 15 standard curves. During the first use, we recommend making 5 µl aliquots of the positive control and storing at -80°C to avoid multiple freeze/thaw cycles. Prepare the standard curve as follows: Dilute 5 µl of the Positive Control with 145 µl Control Diluent to a final concentration of 2.5 µg/ml (see the Quick Chart for Preparing Buffers in this section). Recombinant proteins in solution are temperature sensitive and must be stored at -80°C to prevent degradation. Avoid repeated freeze/thaw cycles and keep on ice when not in storage.

Nu.Q™ 10X Wash Buffer

Prepare the amount of 1X Wash Buffer required for the assay as follows: For every 250 ml of 1X Wash Buffer required, dilute 23.1 ml 10X Wash Buffer with 207.9 ml distilled water (see the Quick Chart for Preparing Buffers in this section). Mix gently by swirling to avoid foaming. The 1X Wash Buffer may be stored at 4°C for up to two weeks.

Nu.Q™ Streptavidin-HRP conjugate

The Nu.Q™ Streptavidin-HRP conjugate is supplied ready-to-use. Use 100 µl per well.

Nu.Q™ Substrate Solution

The Nu.Q™ Substrate Solution should be brought to room temperature before use. The Substrate Solution is light sensitive; therefore, we recommend avoiding direct exposure to intense light during storage. The Substrate Solution should be colorless prior to addition to the wells. A green/blue color present in the Substrate Solution indicates that it has been contaminated and must be discarded. Prior to use, place the Substrate Solution at room temperature for at least 1 hour. After use, discard remaining Substrate Solution.

Nu.Q™ Stop Solution

Is supplied ready-to-use.

Preparing serum samples

This assay is designed to be performed on human serum or K2-EDTA plasma. Do not use icteric, lipidemic or hemolysed sera. Samples containing sodium azide should not be used in the assay. CAUTION: all human specimens should be treated as potential infection hazards.

NOTE: The potential interference of icteric, lipidemic or hemolysed samples in this assay has not been investigated and may affect assay performance. Suspended fibrin particles or aggregates may also affect assay performance. Specimens containing observable particulate matter should be clarified by centrifugation prior to testing. Do not heat the samples.

Specimen Storage

Samples not used immediately may be stored at -20°C for up to 2 months. For long term storage (>2 months) samples should be stored at -80°C. Multiple freeze thaw cycles must be avoided.

Specimen Dilution

The assay should be performed on undiluted serum.

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)
Positive Control Stock	Positive Control	0.333 µl	0.67 µl	4 µl	8 µl
	Control Diluent	9.67 µl	18.33 µl	116 µl	232 µl
	TOTAL REQUIRED	**10 µl**	**20 µl**	**120 µl**	**240 µl**
Detection Antibody	Detection Antibody	0.105 µl	0.84 µl	5.04 µl	10.08 µl
	Detection Diluent	104.9 µl	839.16 µl	5.035 ml	10.07 ml
	TOTAL REQUIRED	**105 µl**	**840 µl**	**5.04 ml**	**10.08 ml**
Streptavidin HRP	**TOTAL REQUIRED**	**105 µl**	**840 µl**	**5.04 ml**	**10.08 ml**
1X Wash Buffer	Distilled water	2.16 ml	17.28 ml	103.7 ml	207.4 ml
	10X Wash Buffer	240 µl	1.92 ml	11.5 ml	23 ml
	TOTAL REQUIRED	**2.4 ml**	**19.2 ml**	**115.2 ml**	**230.4 ml**
Stop Solution	**TOTAL REQUIRED**	**105 µl**	**840 µl**	**5.04 ml**	**10.08 ml**
Substrate Solution	**TOTAL REQUIRED**	**105 µl**	**840 µl**	**5.04 ml**	**10.08 ml**

Read the entire protocol before use.

Determine the appropriate number of microwell strips required for testing samples, controls and blanks in duplicate. Store the unused strips in the aluminum pouch at 4°C. 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 unused wells are stable at room temperature for the duration of the assay if kept dry. Once the assay is finished, unused wells should be returned to the aluminum pouch and stored at 4°C. Unused wells retain activity for up to a month. Use the strip holder while performing the assay.

Allow all buffers to warm up to room temperature before use. Prepare the 1X Wash Buffer as described in the section Buffer Preparation and Recommendations. Multi-channel pipettor reservoirs may be used for dispensing the Complete Assay Buffer, Wash Buffer, Control Diluent, Substrate Solution and Stop Solution into the wells being used.

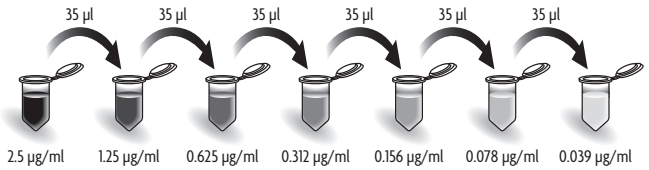
Standard Curve Preparation

Use this plate set-up example to prepare a standard curve for the positive control in duplicate.

	Control											
	1	2	3	4	5	6	7	8	9	10	11	12
A	2.5 ug/ml	2.5 ug/ml	–	–	–	–	–	–	–	–	–	–
B	1.25 ug/ml	1.25 ug/ml	–	–	–	–	–	–	–	–	–	–
C	0.625 ug/ml	0.625 ug/ml	–	–	–	–	–	–	–	–	–	–
D	0.3125 ug/ml	0.3125 ug/ml	–	–	–	–	–	–	–	–	–	–
E	0.156 ug/ml	0.156 ug/ml	–	–	–	–	–	–	–	–	–	–
F	0.078 ug/ml	0.078 ug/ml	–	–	–	–	–	–	–	–	–	–
G	0.039 ug/ml	0.039 ug/ml	–	–	–	–	–	–	–	–	–	–
H	0 ug/ml	0 ug/ml	–	–	–	–	–	–	–	–	–	–

1. The Positive Control is provided at 75 µg/ml. Thaw on ice. Before using, flick the bottom of the tube gently to mix and quick spin the contents to the bottom of the tube. During the first use we recommend making 5 µl aliquots of the stock protein for future standard curves and storing at -80°C to avoid multiple freeze/thaw cycles.
2. Make up a 2.5 µg/ml starting concentration of the Positive Control by adding 5 µl of the 75 µg/ml working stock to 145 µl of Control Diluent and mix by vortexing.
3. Set up the standard curve in duplicate using the following concentrations: 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039 and 0 µg/ml. Each standard curve will require 20 µl of the 2.5 µg/ml starting solution.

4. Pipette 35 µl of Control Diluent into 6 tubes. Use the 2.5 µg/ml starting concentration to prepare a dilution series as indicated below. Add 35 µl of the starting concentration into the 35 µl of Control Diluent in the 1.25 µg/ml tube. Mix gently but thoroughly before each transfer of 35 µl into the next tube. The 2.5 µg/ml standard is the high standard and Control Diluent alone is the 0.0 µg/ml.



Step 1: Binding of Nucleosomes to the Capture Plate

1. Take the carrier tray and the required strips out of the protective pouch.
2. Wash the wells three times with 200 µl 1x Wash Buffer. Following each wash step, remove the solution from the plate by decanting and tapping the plate on an absorbent material (e.g. paper tissue or towel).
3. In duplicate, prepare the amount of desired sample. It is recommended to use undiluted serum or plasma samples.
4. Dispense 90 µl of Assay Buffer into each well to be used including the wells for the standard curve(s).
5. Add 10 µl of each standard curve dilution to the plate wells as outlined in the plate set-up example. Row H will serve as the blank wells with 10 µl of Control Diluent. Unused diluted Positive Control can at be stored at 4°C for up to 1 week.
6. Add 10 µl of undiluted serum sample or 20 µl of undiluted plasma sample to plate.
7. Cover the plate with a sealing film. Press firmly all over the plate to ensure tight seal.
8. Incubate plate containing the standard curve and samples for 2.5 hours at room temperature with agitation on orbital shaker or rocking platform at approximately 700 rpm.

Step 2: Binding of Detection Antibody

9. Before the incubation in Step 1 ends, dilute the Detection Antibody 1:1000 in Detection Diluent and mix thoroughly.
10. Remove the adhesive film. Empty the wells by tapping the plate on an absorbent material.
11. Wash the wells three times with 200 µl 1x Wash Buffer. Following each wash step, remove the solution from the plate by decanting and tapping the plate on an absorbent material.

12. Add 100 µl of Detection Antibody to each well.
13. Cover the plate with a sealing film and incubate at room temperature for 1.5 hours with agitation on orbital shaker or rocking platform at approximately 700 rpm.
14. Repeat the washing procedure in step 10.

Step 3: Binding of Streptavidin HRP

15. Dispense 100 µl of the ready-to-use Streptavidin-HRP Conjugate to each well.
16. Cover the plate with sealing film and incubate at room temperature for 30 minutes with agitation on orbital shaker or rocking platform at approximately 700 rpm. The distribution of the streptavidin-HRP solution can be visually controlled at this step as the streptavidin-HRP solution is yellow in color.
17. During this incubation, place the Substrate and Stop Solution at room temperature.
18. Repeat the washing procedure in step 10.

Step 4: Colorimetric Reaction

19. Remove as much of the final wash as possible by blotting the plate on paper towels.
20. Add 100 µl of room temperature Substrate Solution to all wells being used.
21. Incubate under low or no light conditions for approximately 20 minutes at room temperature under agitation on orbital shaker or rocking platform at approximately 700 rpm (cover the plate with a light-tight cover, do not use adhesive film during this incubation). Please read the Certificate of Analysis supplied with this kit for optimal development time associated with this lot number. Monitor the green/blue color development in the standard curve wells containing the higher concentrations of positive control until they turn medium to dark green/blue. Do not overdevelop.
22. Add 100 µl of room temperature Stop Solution to all the wells to stop the colorimetric reaction. Use the same sequence and rate of distribution as for the substrate solution. Allow perfect homogenization by placing the plate on orbital shaking for 1 minute.
23. Read absorbance on a spectrophotometer at 405 nm. If the OD is read directly after the substrate incubation there is no need to stop the reaction.

Calculation of results using the standard curve

To generate a standard curve using the included positive control, average the duplicate readings for each standard, control, and sample. If preferred the the optical density (OD) obtained from the zero standard (Row H blank wells) may be subtracted from each of the averages.

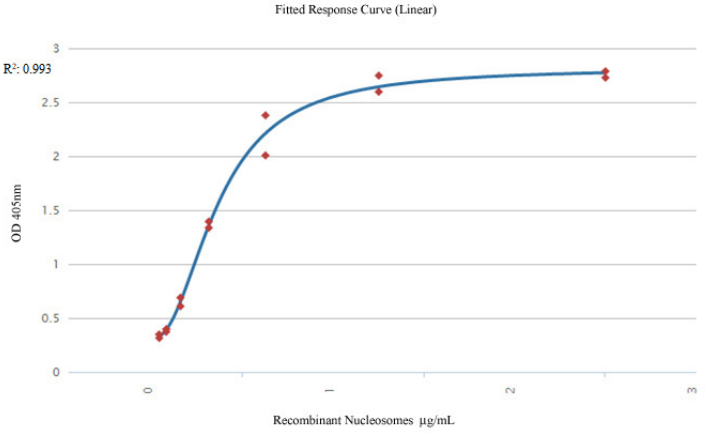
To estimate the amount of circulating H3.1 containing nucleosomes in the samples, plot the OD for the standards against the quantity (µg/ml) of the standards and draw the best fit curve (Microsoft Excel statistical functions can be used for slope calculation or a statistical software such as GraphPad Prism). The best curve fit may vary depending on the developing times used and should be calculated each time a standard curve is run. The data can be linearized using log/log paper or a 4- or 5-parameter logistic regression analysis may also be applied.

Note: If the samples have been diluted, the value read from the standard curve must be multiplied by the dilution factor.

See the example standard curve below.

Example curve:

The following 5-PL standard curve is provided for demonstration only. A standard curve should be made every time an experiment is performed.



Section A: Troubleshooting Guide

Problem/question	Possible cause	Recommendation
No signal or weak signal	Omission of key reagent	Check that all reagents have been added to 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 Substrate Solution and Stop Solution to room temperature before using
	Inadequate volume of Substrate Solution	Check to make sure that correct volume is delivered by pipette
	Developing time too short	Increase the incubation time of the enzymatic reaction
High background in all wells	Developing time too long	Stop enzymatic reaction as soon as the positive wells turn medium-dark green/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 in sample diluent
No signal or weak signal in sample wells	Not enough sample per well	Increase the amount of serum sample per well
	Incorrect sample preparation	Make sure that the samples have been prepared following the recommendations on page 7
No signal or weak signal in standard curve wells	Too many freeze/thaw cycles of protein	During the kit's first use, aliquot the positive control into 5 µl aliquots and store at -80°C to avoid multiple freeze/thaws
	Dilution error	Verify that the serial dilution of the positive control was performed correctly
Large Coefficient of Variation	Presence of bubbles	Ensure no bubbles are present in the wells
	Incomplete resuspension of reagents	Ensure that all reagents, serum samples and buffers, are completely homogenous
	Pipetting error	Use calibrated pipettes
	Inadequate washing	During the wash steps ensure all wells are filled with Wash Buffer and follow washing recommendations

Section B. Related Products

Recombinant Nucleosomes	Format	Catalog No.
Recombinant Polynucleosomes (H3.1)	20 µg	31466
Recombinant Polynucleosomes (H3.3)	20 µg	31468
Recombinant Mononucleosomes (H3.1) - biotinylated	20 µg	31467
Recombinant Mononucleosomes (H3.3) - biotinylated	20 µg	31469
Recombinant Polynucleosomes H3.3 (K27M)	20 µg	31562
Recombinant Polynucleosomes H3.3 (G34L)	20 µg	31558
Recombinant Polynucleosomes H3.3 (G34R)	20 µg	31559
Recombinant Polynucleosomes H3.3 (G34V)	20 µg	31560
Recombinant Polynucleosomes H3.3 (G34W)	20 µg	31561
Recombinant Polynucleosomes H3.3 (K36M)	20 µg	31563
Recombinant Mononucleosomes (H2A.Bbd) - biotinylated	20 µg	31556
Recombinant Mononucleosomes (TH2B) - biotinylated	20 µg	31557
Recombinant Mononucleosomes (H2A.X) - biotinylated	20 µg	31582
Recombinant Mononucleosomes (H2A.Z) - biotinylated	20 µg	31583
Recombinant Mononucleosomes H3K4me3 (EPL) - biotinylated	20 µg	31584
Recombinant Mononucleosomes H3K4me1 (EPL) - biotinylated	20 µg	31585
Recombinant Mononucleosomes H3K9me3 (EPL)	20 µg	31586
Recombinant Histone Octamer (H3.1)	100 µg	31470
Recombinant Histone Octamer (H3.1) - biotinylated	50 µg	31471
Recombinant Histone Octamer (H3.3)	100 µg	31472
Recombinant Histone Octamer (H3.3) - biotinylated	50 µg	31473
Recombinant Mononucleosomes H3K27ac - biotin	10 µg	81002
Recombinant Mononucleosomes H3K4me1/H3K27ac - biotin	10 µg	81003
Recombinant Mononucleosomes H3K4me3/H3K27ac - biotin	10 µg	81004
Recombinant Mononucleosomes H3K4me3/H3K27me3 - biotin	10 µg	81005
Recombinant Mononucleosomes (H3.1)	50 µg	81070
Recombinant Mononucleosomes (H3.3)	50 µg	81071
Recombinant Mononucleosomes H3K36me3 (MLA) - biotin	20 µg	81076
Recombinant Mononucleosomes H4K20me3 (MLA)	20 µg	81080
Recombinant Mononucleosomes H4K20me3 (MLA) - biotin	20 µg	81081
Recombinant Mononucleosomes (H2A.Z)	50 µg	81072
Recombinant Mononucleosomes H3K4me2 (EPL)	20 µg	81073
Recombinant Mononucleosomes H3K4me2 (EPL) - biotin	20 µg	81074
Recombinant Mononucleosomes H3K9ac (EPL)	20 µg	81075
Recombinant Mononucleosomes H3K27ac	10 µg	81077
Recombinant Mononucleosomes H3K36me3 (MLA)	20 µg	81116

DNA Methylation	Format	Catalog No.
Bisulfite Conversion Kit	50 rxns	55016
MethylCollector™ Ultra	30 rxns	55005
UnMethylCollector™	30 rxns	55004
Fully Methylated Jurkat DNA	10 µg	55003
FFPE Bisulfite Conversion Kit	40 rxns	55021
ChIP-Bis-Seq Kit	10 libraries	53048
Global DNA Methylation – LINE-1 Kit	1 x 96 rxns	55017

Nucleosome and Cell-Free DNA (cfDNA) Analysis	Format	Catalog No.
Nucleosome Preparation Kit	20 rxns	53504
Nucleosome Assembly Control DNA	50 µg	53502
NOMe-Seq	10 rxns	54000
Cell-Free DNA (cfDNA) Purification Kit	100 ml	25503

Technical Services

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

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