Chromatin Assembly Kit

Instruction Manual

Catalog # 17-410

Sufficient reagents for 20 chromatin assembly reactions and analyses.

Upon receipt of kit, check individual components for storage conditions. ACF complex, NAP1 protein, and HeLa core histones must be stored at -80°C.

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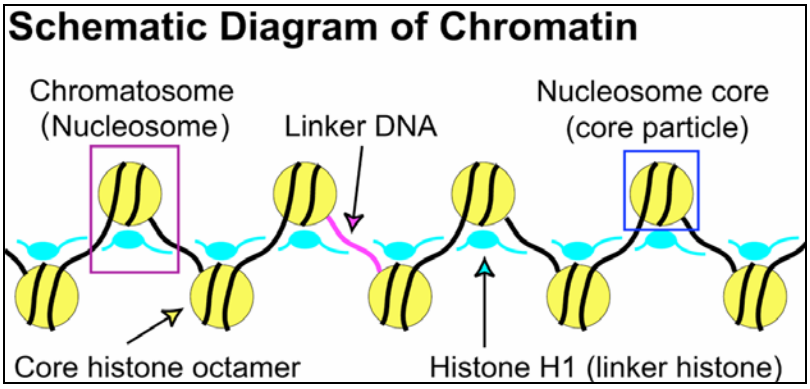
I. INTRODUCTION

A. Background/Overview

Chromatin is the complex of DNA, histones, and other nuclear proteins that condense to form chromosomes. Chromatin condenses to chromosomes during eukaryotic cell division, and forms the higher order structure that allows over a meter of DNA to fit into a single cell nucleus. Thus all essential DNA-protein interactions within the cell, including transcription and replication, depend on and contend with the 3-dimensional structure of chromatin.

Histones are basic, highly conserved proteins that serve as the structural scaffold for the organization of nuclear DNA into chromatin. The four core histones, H2A, H2B, H3, and H4, assemble into an octamer (2 molecules of each). Subsequently, 147 base pairs of DNA are wrapped 1.7 times around the core histone octamer, forming the nucleosome core particle. This 11 nm fiber is often referred to as 'beads on a string'. The linker histone, H1, interacts with linker DNA between nucleosomes and functions in the compaction of chromatin into higher order structures. The chromatosome (nucleosome), is composed of the linker histone H1, linker DNA, and the nucleosome core, and is the basic structural subunit of chromatin.



Histones are modified post-translationally by the actions of enzymes in both the nucleus and cytoplasm. These modifications occur predominantly on the N-terminal and C-terminal tails that extend beyond the nucleosome core particle. The most common modifications are acetylation, phosphorylation, methylation, and ubiquitination. Histone modifications regulate DNA transcription, repair, recombination, and replication, and can alter local chromatin architecture.

Chromatin is the natural state of DNA in the nucleus and all DNA-utilizing processes function in chromatin rather than naked DNA. Research suggests that transcriptional regulation on naked DNA can differ from that on a promoter assembled into chromatin. Thus, it is important to analyze gene regulatory mechanisms in a native, 3-dimensional chromatin state.

The **Millipore Chromatin Assembly Kit** is capable of generating extended, regularly ordered and periodic arrays of nucleosomes *in vitro*. It may be used to study any function of DNA in its native environment. For example:

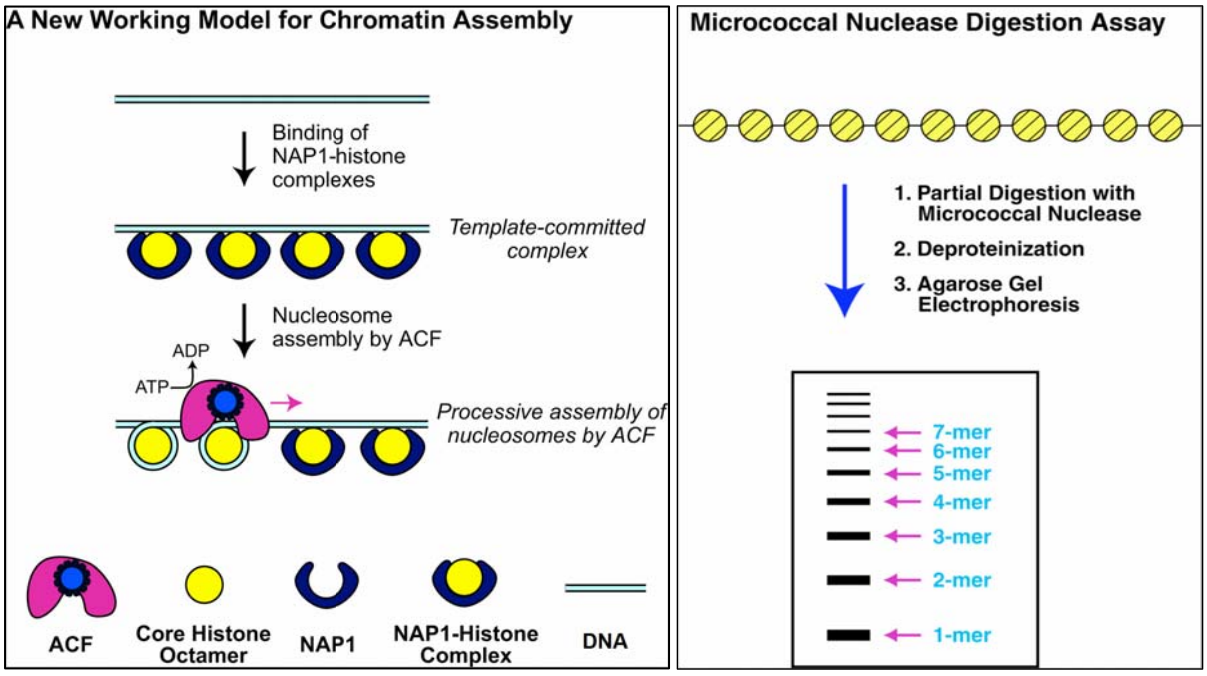
- *In vitro* ChIP assays can be performed to verify transcription factor binding to the assembled chromatin and to investigate cofactor or inhibitor influence on binding.
- Studies of nucleosome core histones modifications such as methylation and acetylation (e.g. *in vitro* HAT Assay).
- *In vitro* Transcription assays can be used to determine factors that are involved in transcription activation from the assembled chromatin.

B. Principle of the Assay

The Chromatin Assembly Kit utilizes purified components for *in vitro* ATP-dependent assembly of extended, regularly ordered and periodic arrays of nucleosomes.

The kit contains the recombinant histone chaperone NAP-1 (Nuclear Assembly Protein-1) and the recombinant Acf1/ISWI complex, which catalyzes the deposition of histones into extended periodic nucleosome arrays in an ATP-dependent manner. The reaction can utilize either circular or linear DNA.

The kit also allows monitoring of the efficiency of the *in vitro* chromatin assembly reaction via a partial digestion of the assembled DNA using micrococcal nuclease. Following deproteinization, the fragmented nucleosomes can be detected as ladders by agarose gel electrophoresis. The nucleosomes can be packed more loosely or more tightly (down to 147 bp repeat length) by varying the assembly reaction conditions. The suggested reaction and micrococcal digestion conditions will produce ordered nucleosome ladders with an apparent repeat length of ~ 147-200 bp.



II. CHROMATIN ASSEMBLY KIT COMPONENTS

A. Provided Kit Components

Each Kit provides sufficient reagents for 20 chromatin assembly reactions and analyses. A single reaction is able to generate 100.0 µl of assembled chromatin from 1.0 µg of DNA.

The dual storage kit contains two boxes, #20-410 (store at -20 °C), and #13-410 (store at -80 °C).

#20-410	Contents (Part 1 of 2)		
Part #	**Reagent**	**Size**	**Storage**
CS202168	Buffer 1	200 µl	-20 °C
CS202169	Buffer 2	1,500 µl	-20 °C
CS202167	Creatine Kinase	25 µl	-20 °C
CS202176	Kinase buffer	200 µl	-20 °C
CS202172	$CaCl_2$	60 µl	-20 °C
CS202173	Enzymatic cocktail	20 µl	-20 °C
CS202166	Enzymatic Stop Solution	700 µl	-20 °C
CS202174	Proteinase K	40 µl	-20 °C
CS202175	Glycogen	40 µl	-20 °C
#13-410	**Contents (Part 2 of 2)**		
Part #	**Reagent**	**Size**	**Storage**
CS202185	ACF complex	10 µg (0.5 mg/ml)	-80 °C
CS202177	NAP1 protein	300 µg (2.5 mg/ml)	-80 °C
CS202178	HeLa core histones	50 µg (1.0 mg/ml)	-80 °C
CS202171	Control DNA	200 µl (0.1 mg/ml)	-80 °C

B. Materials Required But Not Provided

Reagents

- Double distilled, deionized water (ddH_2O)
- SDS (10% solution in ddH_2O)
- Phenol/chloroform/isoamyl alcohol (25:24:1, pH 8.0)
- Ammonium acetate (5 M in ddH_2O)
- Ethanol (100%)
- Ethanol (70% in ddH_2O)
- 5 x DNA dye
- 100 bp DNA ladder
- Ethidium bromide
- TBE buffer
- Liquid nitrogen (for snap-freezing the -80 °C components)

Equipment

- 1.5 ml microcentrifuge tubes (DNase-free & RNase-free). Tubes do not require siliconizing.
- Microfuge
- Variable volume (0.1 – 1,000 µl) micropipettors and tips
- Electrophoresis apparatus
- Timer
- Vortex mixer
- Two variable temperature water baths (27 °C and 55 °C)

III. CHROMATIN ASSEMBLY PROTOCOL

This following protocol involves 2 parts, the chromatin assembly reaction and the analysis to confirm the successful assembly reaction (partial digestion, deproteinization, agarose electrophoresis). The chromatin assembly reaction (Step A) requires 1 to 2 hours on day 1 and an overnight incubation. Analysis (Steps B, C, and D) takes 6 to 8 hours on day 2.

A. Chromatin Assembly Reaction (Day 1 and overnight incubation)

1. The final volume of each chromatin assembly reaction is 100.0 µl.

Note: One chromatin assembly reaction (100.0 µl) requires fixed amounts of **Buffer 1** (10.0 µl) and **ATP Regeneration System** (10.0 µl). **Control DNA** (positive control) can be replaced with **Target DNA** of interest, and **HeLa core histones** (positive control) can be replaced with **Target core histones** of interest. Calculate the amount of **Buffer2** to make the final volume of 100.0 µl. The ratio between DNA and core histones in the *in vitro* chromatin assembly reaction is critical and may require titration for each new DNA and/or histone preparation.

Component	Positive control	Target DNA study	Target core histones study
Control DNA	1.0 µg	**Replaced with target DNA (~1.0 µg)**	1.0 µg
HeLa core histones	1.9 µg	1.9 µg	**Replaced with target core histones (~1.9 µg)**
NAP1 protein	12.5 µg	12.5 µg	12.5 µg
ACF complex	0.5 µg	0.5 µg	0.5 µg
ATP regeneration system	10.0 µl	10.0 µl	10.0 µl
Buffer 1	10.0 µl	10.0 µl	10.0 µl
Buffer 2	to 100.0 µl	to 100.0 µl	to 100.0 µl
Total volume	**100.0 µl**	**100.0 µl**	**100.0 µl**

2. Set one water bath at 27 °C and another at 55 °C.

3. Quick-thaw the following reagents in the 27 °C water bath.
   Buffer 1
   NAP1 protein
   HeLa core histones
   Buffer 2
   Centrifuge briefly to collect material. Then keep reagents on ice.

4. In a new 1.5 ml microcentrifuge tube (tube 1), add the following reagents in order:
   Buffer 1 (10.0 µl)
   NAP1 protein (12.5 µg), (2.5 mg/ml, 5.0 µl)
   HeLa core histones (1.9 µg), (1.0 mg/ml, 1.9 µl)
   Mix by vortexing. Centrifuge briefly to collect material.

5. Incubate on ice for 15 minutes.

6. Add the amount of **Buffer 2** from calculation (62.1 µl for positive control with **NAP1 protein** (2.5 mg/ml, 5.0 µl), **HeLa core histones** (1.0 mg/ml, 1.9 µl), **ACF complex** (0.5 mg/ml, 1.0 µl) and **Control DNA** (0.1 mg/ml, 10.0 µl)). Mix by vortexing. Centrifuge briefly to collect material.

7. Incubate on ice for 15 minutes.

8. Quick-thaw the **ACF complex** in the 27 °C water bath. Centrifuge briefly to collect material. Then keep on ice.

9. Add 0.5 µg (0.5 mg/ml, 1.0 µl) of the **ACF complex** to tube 1. Mix by vortexing. Centrifuge briefly to collect material.

10. After adding **ACF complex**, transfer tube1 from ice to room temperature.

11. Quick-thaw the following reagents in the 27 °C water bath:
    Control DNA
    Kinase buffer
    Centrifuge briefly to collect material. Then keep at room temperature.

12. Quick-thaw the **Creatine Kinase** in the 27 °C water bath. Centrifuge briefly to collect material. Then keep on ice.

13. In a new 1.5 ml microcentrifuge tube (tube 2), make the **ATP Regeneration System** by adding the following reagents in order:
    Kinase buffer (9.9 µl)
    Creatine Kinase (1.1 µl)
    Mix by vortexing. Centrifuge briefly to collect material.

14. Add the following reagents in order to tube 1.

ATP Regeneration System (10.0 µl)
Control DNA (1.0 µg), (0.1 mg/ml, 10.0 µl)
Mix by vortexing. Centrifuge briefly to collect material.

15. Incubate in the 27 °C water bath overnight. The assembled chromatin may be stored at +2-8 °C for up to 2 additional days. Do not freeze.

16. Immediately snap-freeze the vials of the -80°C components in liquid nitrogen prior to re-storage at -80°C. The -20°C components may be stored at -20°C directly. Discard any unused **ATP Regeneration System**.

B. Partial Digestion (Day 2)

17. Quick-thaw the following reagents in the 27 °C water bath.
$CaCl_2$
Enzymatic cocktail
Enzymatic Stop Solution
Centrifuge briefly to collect material. Then keep at room temperature.

18. Centrifuge tube 1 briefly to collect material.

19. Add 3.0 µl of the **$CaCl_2$** to tube 1. Mix by vortexing. Centrifuge briefly to collect material.

20. Incubate in the 27 °C water bath.

Note: The **Enzymatic cocktail** digestion need to be timed precisely.

21. Start a timer and add 1.0 µl of **Enzymatic cocktail**. Mix by vortexing.

22. Incubate in the 27 °C water bath.

23. After exactly 7 minutes, add 34.0 µl of **Enzymatic Stop Solution**. Mix by vortexing. Centrifuge briefly to collect material.

24. The reaction (tube 1) may be stored at -20 °C for later use.

C. Deproteinization (Day 2)

25. If tube 1 is being stored at -20 °C, incubate in the 27 °C water bath to thaw, then move to room temperature.

26. Add 6.9 µl of **10% SDS** solution. Mix by vortexing. Centrifuge briefly to collect material.

27. Quick-thaw the **Proteinase K** in the 27 °C water bath. Centrifuge briefly to collect material. Then keep on ice.

28. Add 2.0 µl of the **Proteinase K**. Mix by vortexing. Centrifuge briefly to collect material.

29. Incubate tube 1 in the 55 °C water bath for 30 minutes. Centrifuge briefly to collect material.

30. Quick-thaw the **Glycogen** in the 27 °C water bath. Centrifuge briefly to collect material. Then keep on ice.

31. Add 2.0 µl of the **Glycogen**. Mix by vortexing. Centrifuge briefly to collect material.

32. Add 51.1 µl of **ddH_2O** to make a final volume of 200 µl. Mix by vortexing. Centrifuge briefly to collect material.

33. Perform a phenol/chloroform extraction by adding 200.0µl of **phenol/chloroform/ isoamyl alcohol (25:24:1, pH 8.0)** to tube 1. Vortex vigorously for 5 minutes. Centrifuge at 14,000 rpm for 6 minutes at room temperature.

34. Carefully remove the top (aqueous) phase containing the DNA and transfer to a new tube (tube 3). Discard tube 1.

35. Add 200.0 µl of **5M Ammonium acetate** to tube 3. Mix by vortexing. Centrifuge briefly to collect material.

36. Add 1.0 ml of 100% **Ethanol**. Mix by vortexing.

37. Centrifuge at 14,000 rpm for 16 minutes at 4 °C. A small white pellet should be visible. Discard the supernatant using vacuum. Be careful not to disturb the pellet.

38. Add 0.5 ml of **70% Ethanol**. Mix by vortexing.

39. Centrifuge at 14,000 rpm for 6 minutes at 4 °C. Discard the supernatant using vacuum. Remove as much of the supernatant as possible.

40. Air dry for 5 minutes.

41. The sample may be stored at -20 °C for later use.

D. <u>Agarose gel electrophoresis (Day 2)</u>

42. Add 8.0 µl of **ddH_2O** to cover the white pellet.

43. Incubate at room temperature for 1 minute.

44. Add 2.0 µl of **5x DNA dye**. Mix by vortexing. Centrifuge briefly to collect material.

45. Perform agarose gel electrophoresis, running a 1.2 % agarose gel at 150 V in cold **TBE buffer** at 4 °C. Use the **100 bp DNA ladder** as a marker.

46. Terminate the electrophoresis when the DNA dye front reaches the desired position of the gel.

47. Stain the gel with **ethidium bromide** (0.75 µg/ml in **ddH_2O**) for 20 minutes, and destain with several changes of **ddH_2O** for 1 to 3 hours.

48. Visualize the gel under UV light.

IV. STORAGE AND HANDLING

A. Precautions

The Chromatin Assembly kit is designed for research use only and not recommended for internal use in humans or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.

B. Storage

This kit is shipped with dry ice. Upon receipt, read the manual thoroughly and check individual components for storage conditions. **ACF complex** (Catalog No. CS202185), **NAP1 protein** (Catalog No. CS202177) and **HeLa core histones** (Catalog No. CS202178) must be stored at -80°C. All other components can be stored at -20°C.

C. Stability

Kit components are stable for 6 months from date of shipment if stored and handled correctly. After initial thaw, the -80°C components are stable for up to 3 freeze-thaw cycles.

D. Handling Recommendations

For maximum recovery of product, briefly centrifuge original vial prior to removing the cap. Rapidly thaw the vial under cold water and immediately place on ice or keep at room temperature, according to the protocol. Aliquot the -80°C components to avoid repeated thawing and freezing. Snap-freeze the vials in liquid nitrogen prior to re-storage at -80°C. The -20°C components can be put back to -20°C directly.

V. REPRESENTATIVE DATA

The Chromatin Assembly Kit utilizes purified components for *in vitro* ATP-dependent assembly of extended, regularly ordered and periodic arrays of nucleosomes. The nucleosomes can be packed more loosely or more tightly (down to 147 bp repeat length) by varying the assembly reaction conditions. The suggested reaction and micrococcal digestion conditions will produce nucleosome ladders with an apparent repeat length of ~ 147-200 bp.

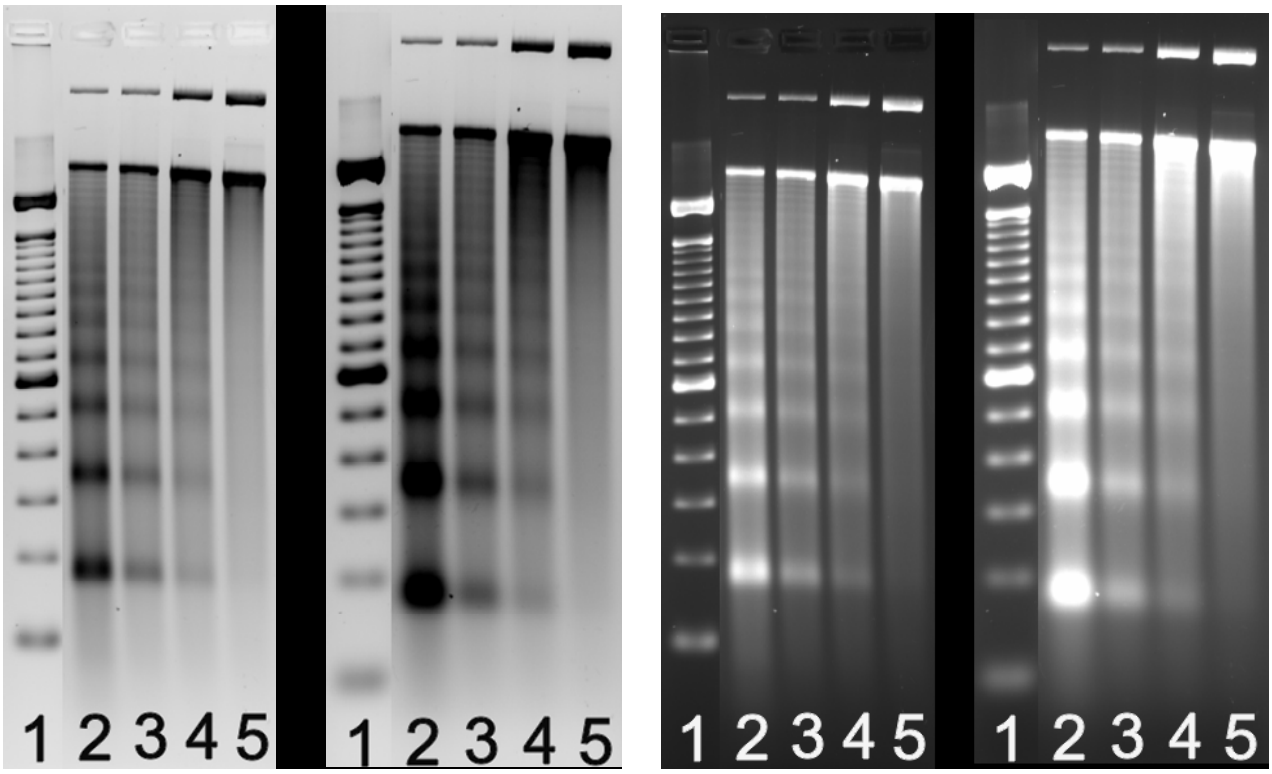


Figure legend
Partial digestion analysis of chromatin assembled with DNA, core histones and recombinant ACF and NAP1 proteins. Titration of DNA and core histones ratios is shown.

Lane 1, 100 bp DNA ladder
Lane 2, DNA (1.0 µg), HeLa core histones (1.9 µg)
Lane 3, DNA (1.0 µg), HeLa core histones (2.1 µg)
Lane 4, DNA (1.0 µg), HeLa core histones (2.4 µg)
Lane 5, DNA (1.0 µg), HeLa core histones (2.9 µg)

VI. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Experimental Suggestions
Less than 6 nucleosome bands on agarose gel without a smear.	The chromatin was over digested.	Use less **Enzymatic cocktail** (step 21) and/or decrease time before adding **Enzymatic Stop Solution** (step 23).
	Too much **DNA** and/or too little **core histones**.	Use less **DNA** and/or more **core histones** (steps 4, 14).
	Sample lost during phenol/chloroform extraction and/or ethanol precipitation	Use tubes with caps that seal tightly (steps 33, 36).
	The **ATP Regeneration System** stopped working.	Use freshly prepared **ATP Regeneration System**. Discard any unused solution (steps 11-14, 16).
Less than 6 nucleosome bands on agarose gel with a smear.	The chromatin was under digested.	Use more **Enzymatic cocktail** (step 21) and/or increase time before adding **Enzymatic Stop Solution** (step 23).
	Too little **DNA** and/or too much **core histones**.	Use more **DNA** and/or less **core histones** (steps 4, 14).
	Low quality DNA	Use high quality **DNA** (step 14) purified using a CsCl gradient or a commercially available DNA purification kit (e.g. Qiagen Plasmid Mini Kit).
	Low quality agarose	Use high quality agarose (step 45).
	Sample did not mix well, especially after adding **core histones** and/or after adding **DNA**	Mix well by vortexing immediately after adding **core histones** (step 4) and after adding **DNA** (step 14).
	The -80°C components, most likely the **ACF complex**, are not working.	After initial thaw, the -80°C components are stable for up to 3 freeze-thaw cycles. Follow **Handling Recommendations** (step 16). Aliquot the -80°C components to avoid repeated freezing and thawing.

VII. REFERENCES

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VIII. RELATED PRODUCTS

Catalog # 14-836	ACF Complex (Acf1/ISWI), active recombinant proteins
Catalog # 14-837	NAP1, active recombinant protein
Catalog # 13-183	Core Histones, HeLa
Catalog # 13-107	Core Histones
Catalog # MAB051	Anti-Histone, core proteins, clone HB-9
Catalog # MAB052	Anti-Histone, H1 + core proteins, clone F152.C25.WJJ
Catalog # 17-295	Chromatin Immunoprecipitation Kit
Catalog # 17-375	EZ-Zyme Chromatin Preparation Kit
Catalog # 17-610	Magna ChIP™ A Chromatin Immunoprecipitation Kit
Catalog # 17-611	Magna ChIP™ G Chromatin Immunoprecipitation Kit
Catalog # 17-408	EZ-Magna ChIP™ A Chromatin Immunoprecipitation Kit
Catalog # 17-409	EZ-Magna ChIP™ G Chromatin Immunoprecipitation Kit

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