

KIT KEY WORDS:

**RELIABLE & CONSISTENT *in vitro* CHROMATIN RECONSTITUTION.
TITRATION GUIDANCE & CONTROLS PROVIDED.**

**NOVEL STEP-by-STEP METHOD. C.A. PURIFICATION PROTOCOL INCLUDED.
VALIDATED USE IN MAJOR *in vitro* APPLICATIONS.
20 C.A. reactions/kit, each involving 2 µg of DNA.**

C.A. Kit

In vitro Chromatin Assembly Kit

Catalog #: ca-vitro-001

❖ **DNA module for C.A.:** catalog # ca-vitro-002 (one additional module)

Instruction Manual

(version 01)

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1. INTRODUCTION

The DNA in eukaryotes is packaged into chromatin, of which the individual chromatin subunits—the nucleosomes—are composed of 147 bp of DNA wrapped almost twice around the core histone octamers. The octamers are composed of two copies each of the four core histone proteins H2A, H2B, H3, and H4 (Wolffe, 1998). Besides its structure-related function, that is, the compaction of DNA, **chromatin** also regulates various aspects of DNA metabolism, including transcription. This is feasible because chromatin exists in a dynamic state (Loyala et al., 2003a).

The structure of cellular chromatin is capable of undergoing rapid changes to respond to the metabolic requirements of the cell. These changes have a direct impact on **gene expression** and, therefore, the chromatin context must be considered when biochemical reactions that involve DNA are studied. DNA-binding factors have evolved with chromatin. It is therefore more desirable to study the molecular mechanisms of DNA-directed processes with chromatin than with naked DNA templates (Lusser and Kadonaga, 2004). To this end, it is necessary to reconstitute DNA and histones into chromatin. Here, we present a **novel kit for the reconstitution of chromatin *in vitro***.

Over the past several decades, a number of methods for assembling chromatin *in vitro* have been described. Some of them use chemical compounds to deposit histone octamers onto the DNA. Others take advantage of cellular protein complexes that have the ability to assemble chromatin. Some of these complexes have been identified and purified (Loyala et al., 2003b). Several histone chaperones have been identified and their role is to guide histone proteins to DNA. Moreover several chromatin assembly factors have been identified and, generally, consist of multiple proteins (Leroy et al., 1998).

The **remodeling and spacing factor (RSF)** has adenosine-triphosphate dependent nucleosome remodeling and spacing activity (Leroy et al., 1998). **The nucleosome assembly protein 1 (NAP-1)** is a histone chaperone. Both RSF and NAP-1 are used to reconstitute chromatin *in vitro* (see kit description and protocol). The advantage of **the RSF-mediated chromatin assembly reaction** over previous described techniques (e.g. salt dialysis, polyglutamic acid treatment and use of *Drosophila* and *Xenopus* extracts) is the formation of regulatory spaced chromatin in a highly defined system.

The **ratio between DNA and core histones** in the *in vitro* chromatin assembly reaction is critical and needs to be **titrated** for every new DNA and histone preparation. If the ratio is not correct, chromatin assembly will fail (see our control strategy described in our kit protocol at STEP 1).

The **efficiency of the chromatin assembly** reaction can be evaluated by partial micrococcal nuclease (MNase) digestion or electron microscopy (see STEP 2 of our kit protocol and results section).

In vitro chromatin assembly can be used to decipher subtle chromatin alterations that play a regulatory role in DNA metabolism (Loyala et al., 2003b). Reconstitution of recombinant chromatin allowed to directly study the role of different histone modifications in the chromatin assembly reaction and in transcription (Loyala et al., 2001).

The Novel Diagenode C.A. kit is designed to perform *in vitro* Chromatin Assembly (C.A.) and C.A. analysis. The assembled chromatin is then submitted to purification, and subsequent *in vitro* tests.

This **brand new *in vitro* Chromatin Assembly method** provides you with **controls** to be used in parallel with your DNA sample. The C.A. **titration optimization** protocol is also given allowing you to work with your DNA sample using the **best proteins:DNA ratio** for optimum *in vitro* C.A..

This kit method introduces you to four inevitable C.A. steps:

STEP 1/

the C.A. experiment is first submitted to a **titration step** for **QUALITY control**,

STEP 2/

C.A. reactions are submitted to **MNase digestion** for **EFFICIENCY control**,

STEP 3/

once the C.A. experiment is set up, **the “final” optimized C.A.** can take place,

STEP 4/

then the ***in vitro* assembled chromatin is purified**.

These steps are three essential keys for RELIABLE and CONSISTENT results.

In the next few pages of this manual you will find the kit method overview, the kit content and the detailed kit protocol. A troubleshooting guide and additional protocols are also present at the end of the manual.

2. KIT METHOD OVERVIEW

In vitro C.A.

Histones
+ RSF + NAP-1
+ 10x complete ATP mix
+ Buffers

+ DNA
(Ctrl or sample(s))

RSF: human, recombinant
(baculovirus)

NAP-1: mouse, recombinant
(baculovirus)

Histones: native histone
octamers (HeLa cells)

1. C.A. titration (Histones versus DNA sample)



2. Analyse the C.A. by MNase



Agarose gel

3. C.A. (using best titer)



4. Purification



in vitro applications

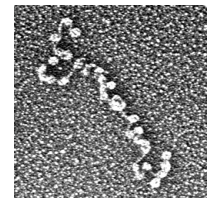
ChIP



Transcription



E.M.



The C.A. kit from Diagenode is designed for *in vitro* Chromatin Assembly (C.A.).

The content of the C.A. kit is more than sufficient to perform 20 Chromatin Assembly assays including C.A. titration and C.A. analysis. Each C.A. assay requires 2 μ g of DNA. Optimized buffers and protocols are provided to perform C.A. titration (STEP 1) and C.A. analysis (STEP 2). The first two steps are used to determine the best proteins:DNA ratio for C.A. to be used with your DNA sample. Then at STEP 3, the optimized C.A. reaction is repeated. Finally, the resulting assembled chromatin undergoes subsequent purification (STEP 4). Protocols are provided for C.A. purification (STEP 4) and *in vitro* tests such as *in vitro* ChIP, transcription and electron microscopy (E.M.).

Note that a module including three buffers for the preparation of DNA to be used in C.A is available (DNA module for C.A., see content and protocol below).

C.A. Kit: Protocol Steps and Time Table.

The C.A. kit can be purchased with an additional module to be used for the DNA sample preparation. Each, kit and module, is provided with adapted buffers and detailed protocols.

FIRST PREPARE DNA ready-to-C.A.:

Table 1a

DNA MODULE for C.A.		DAY	Time needed
-	-	-	Several Days

DNA module for C.A. is composed of three buffers to be used with an optimized protocol for the preparation of DNA ready-to-use in chromatin assembly.

The preparation of DNA ready-to-C.A. takes several days. Although time-consuming, we highly recommend the use of CsCl purified DNA. Alternatively quicker methods can be used to prepare your DNA sample, but the DNA quality will significantly decrease and that will have an impact on the C.A. efficiency (e.g.: the plasmid purified by Qiagen and Sigma kits can show poorer chromatin assembly).

AND THEN PROCEED TO Chromatin Assembly (C.A.):

Table 1b

Chromatin Assembly (C.A.) KIT		DAY	Time needed
STEP 1-	Titration	DAY 1	1 hour + O.N. incubation
STEP 2-	MNase analysis	DAY 2	Few hours hand-on-work
STEP 3-	C.A. (using best titer)	DAY 2	1 hour + O.N. incubation
STEP 4-	Assembled chromatin purification	DAY 3	About 2 hours

In the C.A. kit, the necessary proteins are provided as well DNA control. The C.A. has been optimized to get reliable results: by the use of our reagents, buffers and protocol. The C.A. quality and efficiency can indeed be optimum by performing initial C.A. titration and MNase digestion after C.A. reaction (STEPS 1 and 2 respectively).

The C.A. titration is performed in about one hour on day 1 followed by an overnight incubation (STEP 1). Beginning of day 2, the C.A. is analysed (STEP 2). Next, end of day 2 and using the best titer (Histones:DNA ratio), the “final” C.A. can be performed (STEP 3). In addition, the essential purification step protocol to be performed on day 3 is given (STEP 4). The purification of assembled chromatin leads the researcher to subsequent *in vitro* test results of guaranteed quality (see application section with protocols, results and references).

One format: 20 C.A. rxns per kit (2 µg of DNA /rxn)

3. KIT MATERIALS

Kit Content

The content of the kit is sufficient to perform 20 **Chromatin Assembly assays** (C.A.): from C.A titration to C.A. analysis (C.A. kit: cat #ca-vitro-001). Each C.A. assay requires 2 µg of DNA. Buffers for the preparation of DNA ready-to-C.A. are separately available, see below (DNA Module for C.A.: cat# ca-vitro-002).

The kit and module content is described in Table 2.

Upon receipt, aliquot and store the components as indicated.

SOME REAGENTS SHOULD BE ALIQUOTED AND FROZEN AT -80°C AS INDICATED IN TABLE 2.

Required Materials Not Provided

Reagents

- Gloves to wear at all steps
- Autoclaved tips
- RNase/DNase-free 1.5-ml tubes (the use of siliconized tubes is totally optional)
- Water
- Phenol/chloroform/isoamyl alcohol (25:24:1)
- Chloroform/isoamyl alcohol (24:1)
- Ethanol 100%
- Ethanol 70%
- Agarose and TBE buffer
- DNA molecular weight marker (e.g. 123 bp ladder)
- 6x loading dye
- Ethidium bromide-water bath (1 µg/ml)

Equipment

- Centrifuges for 1.5-ml tubes (4°C), 15-ml and 50-ml tubes
- Fume hood
- Vortex
- Water bath (30°C, 37°C)
- Agarose gel apparatus

Upon receipt, aliquot and store as indicated.

Table 2a: (cat #: ca-vitro-002)

DNA Module for C.A.			
Component	Comments	Quantity	Storage
Buffer 1	Ion chelator included	50 ml	4°C
Buffer 2	Detergent included	150 ml	RT
Buffer 3	Acid included	100 ml	RT

Table 2b: (cat #: ca-vitro-001)

Chromatin Assembly (C.A.) KIT			
Component	Comments	Quantity	Storage
Histones	<u>to be aliquoted in 5 tubes</u> (14 µl/tube)	75 µl	-80°C
Blocker	5 mg/ml	120 µl	-20°C
RSF and NAP-1	<u>to be aliquoted in 5 tubes</u> (16 µl/tube)	90 µl	-80°C
Buffer A	Salt and ion chelator included	6 ml	-20°C
Buffer B	Salt included	500 µl	RT
10x ATP Mix-1	Salt included	330 µl	-20°C
10x ATP Mix-2	-	60 µl	-20°C
10x ATP Mix-3	Salt included	40 µl	-80°C
DNA Control	0.2 µg/µl	60 µl	-20°C
MNase	100 mU/µl	30 µl	-80°C
Buffer C	MNase Start buffer	150 µl	RT
Proteinase K	20 mg/ml	30 µl	-20°C
Buffer D	MNase Stop buffer	6 ml	RT
Co-precipitant	-	75 µl	-20°C
Precipitant	-	600 µl	RT

4. KIT ASSAY PROTOCOL

C.A. KIT from Diagenode for in vitro Chromatin Assembly

Starting material-

Each C.A. assay requires 2 µg of DNA; scale accordingly.

Prior to C.A.:

DNA samples are first prepared as described below.

The **DNA module for C.A. from Diagenode** provides you with three main buffers as well as an optimized protocol for the preparation of DNA ready to use in C.A.. Note that the complete list of additional reagents not provided is given below as well as a list of required equipment. The method of DNA preparation consists on the purification of closed circular DNA by equilibrium centrifugation in CsCl-ethidium bromide gradients.

Required Materials Not Provided

Reagents

- Gloves to wear at all steps
- Autoclaved tips
- Bottles and tubes as described in the protocol below
- Culture medium, antibiotic(s)
- Isopropanol, NH₄Ac, TE, CsCl, Ethidium Bromide, Isoamyl alcohol, Water
- Ethanol 70%
- 19-gauge needle

Equipment

- Ultracentrifuges and centrifuges (see protocol below)
- Fume hood

Protocol

1. Pick a single bacterial colony from a plate and transfer it into 50 ml of **LB medium** containing the appropriate **antibiotic** in a 250-ml flask. Incubate the **pre-culture** overnight (O.N.) at 37°C with vigorous shaking.
2. Inoculate a 4-litre Erlenmeyer flask filled with **1 litre** of LB medium containing the appropriate antibiotic with 10 ml of pre-culture. Incubate the **culture** overnight at 37°C with vigorous shaking. (Work with 2 litres to 10 litres of culture in total).

3. Centrifuge the culture in a 1-litre bottle in a Beckman J-6B centrifuge at 4,000 rpm for 30 minutes, at 4°C. Pour off supernatant carefully, place bottle with upside down on paper towels to remove all the medium.
 4. Add to the pellet 20 ml of **Buffer 1**. *-per litre of culture-*. Resuspend the pellet completely in the buffer by vigorous vortexing until no cell clumps remain.
 5. Add 60 ml of **Buffer 2**. *-per litre of culture-* and mix gently by inverting 4-6 times and incubate for 5 minutes on ice. Do not vortex to avoid shearing of genomic DNA. The lysate should appear viscous.
 6. Add 45 ml of **Buffer 3**. *-per litre of culture-*, mix immediately but gently by inverting 4-6 times and incubate on ice for 30-60 minutes.
 7. Centrifuge in a 250-ml GSA bottle at 11,000 rpm for 60 minutes. Collect the supernatant containing DNA in a fresh bottle.
 8. Precipitate the double-stranded DNA by adding 125 ml of **isopropanol** *-per 1 litre of culture-*. Mix by vortexing and leave it for 5 minutes at room temperature.
 9. Centrifuge at 10,000 rpm for 30 minutes at 4°C. Decant the supernatant carefully (the isopropanol pellet has glassy appearance and may be difficult to see). Place the bottle in an inverted position on a paper towel to allow all of the fluid to drain away.
 10. Resuspend the pellet in 10 ml of **2 M NH₄AC** *-per litre of culture-*, leave it on ice for 5 minutes (RNA, Protein, ... will be removed). Spin at 7,000 rpm for 15 minutes. Transfer the supernatant into fresh 50-ml Falcon tube.
 11. Add 1.0 volume of **isopropanol** to the supernatant. Centrifuge at 10,000 rpm for 20 minutes. Wash the pellet with cold ethanol 70% and centrifuge at 20,000 x g for 15 minutes. Dry the pellet.
 12. Resuspend the pellet in 8 ml **1xTE** and add **8.8 g CsCl** *-per 1 litre of culture-*. After CsCl is dissolved, add 0.3 ml of 10 mg/ml **ethidium bromide** and dissolve completely. It takes a while for CsCl to go into the DNA suspension.
 13. Fill the suspension into a Beckman Optiseal™ polyallomer centrifuge tube (26 x 77 mm, 32.4 ml). Fill up the whole tube with the DNA/CsCl suspension, don't let air bubbles get stuck in the tube and seal the tube with a Beckman tube cap.
- ** Caution: Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn when working with solutions that contain this dye.*
14. Weight the tubes and balance them within 0.02 g, then put the tubes into a 70.1 Ti rotor and don't forget to put the adaptors on top of centrifuge tubes.

15. Centrifuge at 53,000 rpm for more than 18 hours at 20°C.

*** Two bands of DNA, located in the center of the gradient, should be visible in ordinary light. The upper band, which usually contains less material, consists of nicked/relaxed circular plasmid DNA; the lower band consists of closed circular plasmid DNA. The deep red pellet on the bottom of the tube consists of ethidium bromide/ RNA complexes.*

16. Collect the bands of DNA with a 19-gauge needle in minimal volume, usually about 1 ml. Remove the cap from the tube to allow air to enter before sucking out the sample.

*** Ethidium Bromide will be removed from the DNA with isoamyl alcohol (see below).*

17. Prepare a solution of 1:1 isoamyl alcohol and water and collect the upper phase.

18. Add an equal volume of **saturated isoamyl alcohol** to the DNA sample, mix the two phases by vortexing and centrifuge the mixture at 5,000 rpm for 2 minutes. Decant the upper phase (pink) and keep the lower phase.

19. Repeat the extraction 4-5 times until all the pink colour disappears.

20. Transfer the lower phase into a clean tube (measure the volume, 1x V). Add 3 volumes of 1xTE and mix. Final volume is 4x V.

21. Add 1 volume of the final volume (4x V) of **isopropanol** to the DNA suspension, centrifuge at 8,000 rpm for 20 minutes.

21. Wash the DNA pellet with 1 volume **ethanol 70%**. Dry the pellet at room temperature.

22. Redissolve the DNA in 1 ml of **1xTE** and measure the DNA concentration.

23. Store the DNA at -20°C.

The expected yield is of 1-2 mg DNA per litre of culture.

In vitro Chromatin Assembly (C.A.)

DNA is submitted to C.A. reactions including controls, titration (STEP 1) and assembled chromatin analysis (STEP 2). The best C.A. conditions are used again (STEP 3) to prepare assembled chromatin, which is then purified (STEP 4).

STEP 1: Chromatin Assembly titration (to determine the best histones:DNA ratio)

Optimization of C.A. conditions, including controls

STEP 2: Chromatin Assembly analysis

MNase digestion

STEP 3: Chromatin Assembly (with best histones:DNA ratio)

“Final” optimized C.A reaction using your DNA sample

STEP 4: Assembled chromatin purification

STEP 1: Chromatin Assembly titration

C.A. conditions Overview

* The C.A. incubation mix consists of histones, RSF and NAP-1 together with DNA in buffers including ATP mix (see Table 3 below).

Table 3: C.A. incubation mix components OVERVIEW

#	Reagent	Volume	Comments
1	Buffer A	110.10 µl	[150.00 - x] µl x is the total volume of reagents #2 to #7 Add Buffer A to reach a final volume of 150.00 µl
2	10x complete ATP mix	15.00 µl	
3	Histones	2.00 µl	Variable when titrating your DNA sample (see Table 5.)
4	Blocker	3.00 µl	
5	RSF and NAP-1	3.50 µl	Not to include in the -ve Control
6	Buffer B	6.40 µl	
7	DNA	10.00 µl	Use 2 µg of DNA per rxn, (2 µg/10 µl) Include the kit DNA in the +ve Control Use your DNA sample next to the +ve Control
	TOTAL VOLUME	150.00 µl	

STEP 1 quick chart and detailed protocol are given in the next pages.

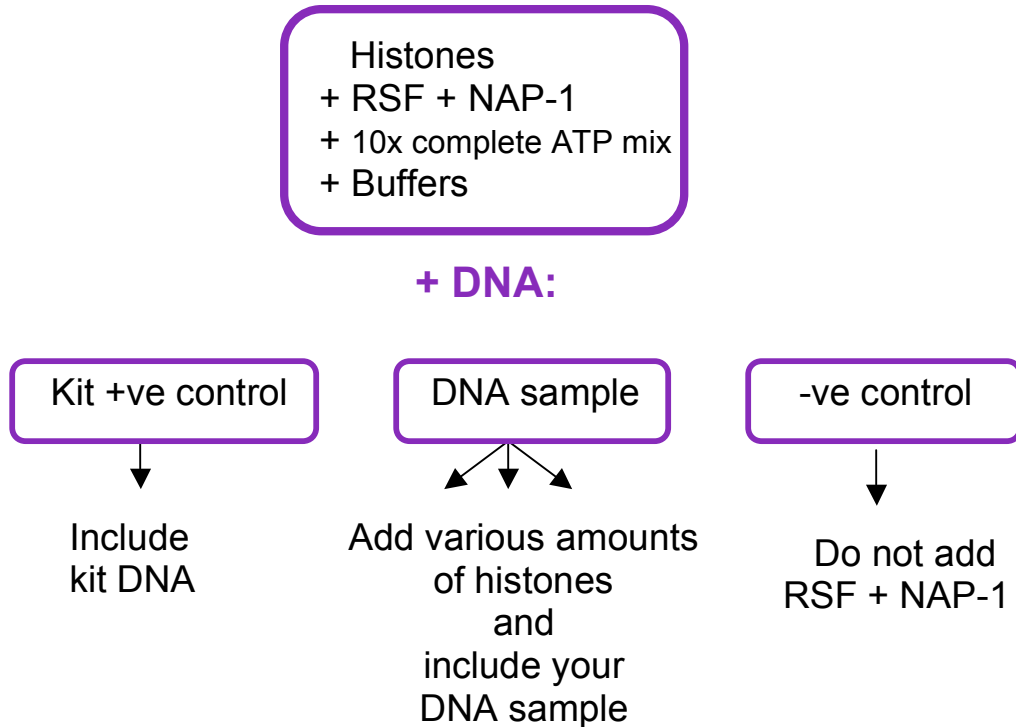
* First, prepare the 10x complete ATP mix (Table 4).

* Then proceed to the C.A. **titration** step (consisting in the use of three -increasing- amounts of histones per reaction) (Table 5).

* Include positive and negative **controls** in your experiment (Table 5).

Quick chart: STEP 1

in vitro Chromatin Assembly titration



Notes about the number of reactions to perform per assay:

- ❖ **Kit controls:** When using the kit DNA as positive control together with the negative C.A. rxn control, you do 2 reactions.
- ❖ **Titration:** If testing your DNA in C.A. titration together with the kit controls (+ve and -ve), you will do 5 reactions.
- ❖ **Final C.A.:** To perform a Chromatin Assembly in duplicate with the best condition (determined by titration), you will do 2 reactions.

First, prepare the 10x complete ATP Mix

- Thaw on ice the reagents listed in the following Table 4. Add one by one to a new 1.5-ml tube the reagents as listed in Table 4 to prepare the 10x complete ATP Mix.
 - ❖ We recommend the use of fresh complete buffer for each new C.A. experiment. Prepare what is needed on the day and discard what is not used.
 - ❖ Alternatively you can prepare more buffer, aliquot and freeze at -80°C . Avoid multiple freeze thawing.

Table 4: 10x Complete ATP Mix

	One reaction	2 rxns + 10% excess	5 rxns + 10% excess
10x ATP Mix-1	9.00 μl	19.80 μl	49.50 μl
10x ATP Mix-2	1.50 μl	3.30 μl	8.25 μl
10x ATP Mix-3	1.00 μl	2.20 μl	5.50 μl
Buffer A	3.50 μl	7.70 μl	19.25 μl
Total volume	15.00 μl	33.00 μl	82.50 μl

- Tap the tube side to mix. Keep on ice until use.

Perform the C.A. titration

- Thaw on ice the reagents listed in the following Table 5.

Table 5: C.A. titration experiments

C.A. Mix	Positive Ctrl	DNA sample (low)	DNA sample (medium)	DNA sample (high)	Negative Ctrl
Buffer A	110.10 μl	110.50 μl	110.30 μl	110.10 μl	113.60 μl
10 x Complete ATP Mix	15.00 μl	15.00 μl	15.00 μl	15.00 μl	15.00 μl
Histones	2.00 μl (1.80 μg)	1.60 μl (1.44 μg)	1.80 μl (1.62 μg)	2.00 μl (1.80 μg)	2.00 μl
Blocker	3.00 μl	3.00 μl	3.00 μl	3.00 μl	3.00 μl
RSF+NAP-1	3.50 μl	3.50 μl	3.50 μl	3.50 μl	-
Buffer B	6.40 μl	6.40 μl	6.40 μl	6.40 μl	6.40 μl
DNA	10.00 μl KIT DNA	10.00 μl	10.00 μl	10.00 μl	10.00 μl
TOTAL VOLUME	150.00 μl	150.00 μl	150.00 μl	150.00 μl	150.00 μl

- Label 1.5-ml tubes. Label as follows for the complete set of 5 reactions.
 - ❖ #1: Positive Ctrl.
 - ❖ #2: DNA sample with low amounts of histone core.
 - ❖ #3: DNA sample with intermediate amounts of histone core.
 - ❖ #4: DNA sample with high amount of histone core.
 - ❖ #5: Negative Ctrl.

5. Add to the labeled tubes one by one the reagents as listed in Table 5. above. When all the reagents are added except for the DNA, mix by taping gently on the tube, then add the DNA, mix again by taping on the tube.
 - ❖ *Each time you add a new reagent, add it directly to the solution and pipette up and down to mix.*
 - ❖ *Change tips each time you add one new reagent to a new tube.*
 - ❖ *Keep all the reagents on ice.*
 - ❖ *Keep samples at room temperature (RT, benchtop).*
6. Spin down, at low speed: 500 x g (3,000 rpm) for 10 seconds.
7. Incubate at 30°C in a water-bath overnight.
 - ❖ *It is possible to do a shorter incubation, however that can't be less than 6 hours. We therefore highly recommend the overnight incubation.*

STEP 2: Chromatin Assembly analysis

MNase digestion

8. Thaw on ice the **MNase** and **Proteinase K**.
Place **Buffer A** and **Buffer C** on ice as well.
Buffer D should be warmed up (37°C for a while) before use.
9. Label two new 1.5-ml tubes ("a" and "b"). Prepare two dilutions of MNase as follows:
 - ❖ *"a" 1:40 dilution of the provided MNase stock solution.*
 - ❖ *"b" 1:20 dilution of the provided MNase stock solution.*
 - ❖ *Prepare the volume of diluted MNase needed on the day. Scale volume accordingly (see the two tables below). Volumes are given for one C.A. reaction, two or five.*
 - ❖ *Keep on ice until use.*

Table 6: Diluted MNase "a".

	One reaction	2 rxns + 10% excess	5 rxns + 10% excess
MNase Stock	0.25 µl	0.55 µl	1.37 µl
Buffer A	9.75 µl	21.45 µl	53.63 µl
Total volume	10.00 µl	22.00 µl	55.00 µl

Table 7: Diluted MNase "b".

	One reaction	2 rxns + 10% excess	5 rxns + 10% excess
MNase Stock	0.50 µl	1.10 µl	2.75 µl
Buffer A	9.50 µl	20.90 µl	52.25 µl
Total volume	10.00 µl	22.00 µl	55.00 µl

10. Label new 1.5-ml tubes (same amount of tubes as used at *Point 4.* above).
 - ❖ *Using the same #, add the letter "b" after the numbers used at Point 4. above.*
 - ❖ *#1b, #2b, #3b, #4b, #5b.*

11. Take your C.A. samples out of the 30°C water bath (from *Point 7.*).
12. Transfer 75 µl of the C.A. reaction from one tube to the corresponding new labeled “b” tube: e.g.: #1b, #2b, #3b, #4b, #5b (prepared at *Point 10.*). Keep the other half of the reaction in the original tubes (e.g.: #1, #2, #3, #4, #5).
13. Add per tube: 10 µl of diluted MNase “a” or “b”. Tap gently on the tube wall to mix.
 - ❖ Add the MNase “a” in the tubes labeled: e.g. #1 to #5. See Table 8 below.
 - ❖ Add the MNase “b” in the tubes labeled: e.g. #1b to #5b. See Table 8 below.
 - ❖ Keep the tubes at RT (benchtop).
14. Then, go next to the 30°C water bath with your sample tubes, the **Buffer C** and a timer.
 - ❖ You will also need pipette and tips.
15. Set the timer at 10 minutes. Add 2.30 µl **Buffer C** per tube, each 10 seconds. Gently tap the tube wall to mix.
 - ❖ Order the tubes and add buffer to tubes in a precise order: from first to last tube (e.g.: from tube #1 to #5 then #1b to #5b).
 - ❖ Correct and precise timing is essential.
 - ❖ Make fixed intervals of 20 seconds if you prefer (as long as intervals are regular).
 - ❖ This is to start the enzymatic reaction.

Table 8: MNase reactions (digestion of the assembled chromatin).

	MNase “a”	MNase “b”
C.A. rxn (<i>Point 7.</i>)	75.00 µl	75.00 µl
Diluted MNase (<i>Point 9.</i>)	10.00 µl	10.00 µl
Buffer C	2.30 µl	2.30 µl
Total volume	87.30 µl	87.30 µl

16. Incubate all the tubes for precisely 10 minutes at 30°C each.
17. Then, next to the water bath, add again each 10 seconds, 88 µl of **Buffer D** per tube. Gently tap the tube wall to mix.
 - ❖ Start with the same tubes as above: e.g. from first to last tube (tubes #1 to #5 then #1b to #5b).
 - ❖ There is no need to change tips, as long as you do not touch the tube walls when adding the buffer to each tube.
 - ❖ Correct and precise timing is essential.
 - ❖ Make fixed intervals of 20 seconds if you prefer (use same interval time as at *Point 5.*).
 - ❖ This is to stop the enzymatic reaction.
18. Back at the bench, add 1 µl of **Proteinase K** per tube.
 - ❖ Add the proteinase directly in the sample, pipette up and down a few times to mix.
 - ❖ Close tube and gently tap to mix.
 - ❖ Use a new tip for each sample tube.
 - ❖ While adding the Proteinase K, keep all the tubes at RT (benchtop).

19. Incubate for 30 minutes in a 37°C water bath.
❖ *It is possible to incubate up to one hour, but this is not necessary.*

DNA extraction

20. Add 176 µl (1 volume) of phenol/chloroform/alcohol isoamyllic per tube.
❖ *Work under fume-hood.*
21. Vortex vigorously for 30 seconds.
22. Centrifuge at 14,000 x g (12,000 rpm, or maximum speed) for 5 minutes, at RT.
23. Meanwhile, place on ice the **co-precipitant** and **precipitant**. Label new 1.5-ml tubes.
❖ *e.g. tubes #1 to #5 and #1b to #5b.*
24. In the new labeled tubes, add 1 µl of **co-precipitant**. Then, add 10 µl of **precipitant** to each tube.
25. After the centrifugation (*Point 23.*), transfer the top layer to the new tubes (*Point 24.*). Gently tap to mix. Work under fume-hood.
26. Add 600 µl ethanol 100% per tube.
27. Incubate at -80°C for 30 minutes.
28. Meanwhile, prepare a 1.3% agarose gel. Use fresh TBE buffer.
❖ *It is important to work with fresh reagents.*
❖ *We recommend the use of a 13 cm (up to 20 cm long) gel.*
29. Centrifuge at 14,000 x g (12,000 rpm, or maximum speed) for 10 minutes, at 4°C.
30. Discard the supernatant. Keep the pellet.
31. Add 600 µl ethanol 70% to each pellet.
32. Centrifuge at 14,000 x g (12,000 rpm, or maximum speed) for 10 minutes, at 4°C.
33. Discard the supernatant. Air-dry the pellets at RT for a while.
34. Add 10 µl water to each pellet.
❖ *This is the DNA purified from the C.A. samples.*
35. Add 2 µl of 6x loading dye to each tube. Gently tap to mix. Resuspend.
❖ *All of the DNA sample is used for analysis.*
36. Centrifuge at 3,000 rpm for about 30 seconds, at RT.

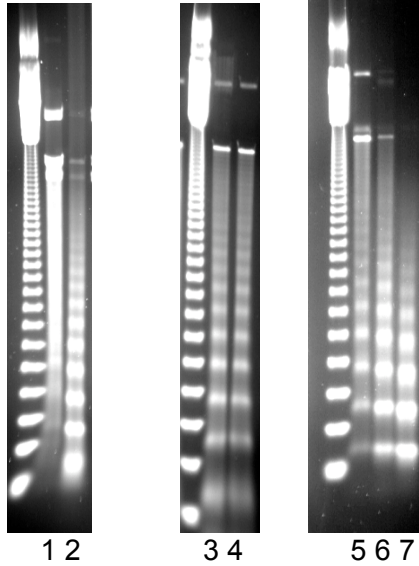
DNA analysis on agarose gel

37. Add a MW marker (5 μ l per well of 123 bp ladder) on each side of the 1.3% agarose gel.
38. Load the DNA samples on the gel.
39. Run at 150 V at RT. Stop the run when the blue gel migration front reaches 2-3 cm from the bottom of the gel. (In our conditions, that should take about 2 hours)
40. Incubate in an ethidium bromide-water bath for 30 minutes. That is to stain the DNA.
41. Incubate the agarose gel in water for another 30 minutes (up to one hour). That is to destain.
42. Under UV light, look at the gel and make a picture (see Figures 1. and 2. below)

Analysis of the C.A. titration: expectations and what to do next

43. Analyse the digestion on gel. Below some major comments and gel pictures are also given as examples of C.A. analysis.
 - ❖ *The C.A and digestion can both be good, if so, then proceed to next STEP.*
 - ❖ *The C.A can be fine, but chromatin over-digested.*
If the chromatin is over-digested, showing the lower bands with high intensity, then you could perform a new C.A. and then digest the samples with lower amounts of MNase: 10.00 and 25.00 mU/ digestion instead of 25.00 and 50.00 mU/ digestion (“a” and “b” respectively, Point 9).
 - ❖ *If no condition is satisfactory, do another titration again using lower or higher ranges of histone amounts (depending on the results). See Troubleshooting guide.*
44. Choose the amount of protein that gives you the best Assembled Chromatin and proceed to final Chromatin Assembly at STEP 3.
 - ❖ *We highly recommend the use of CsCl purified DNA and the use of all the reagents provided with the C.A. kit. It is also as suggested in the protocol very important to include controls in your experiments and to perform the C.A. titration (STEP 1) before starting the “final” C.A. experiment. The final optimized C.A. (STEP 3) will then be done with the best proteins:DNA ratio (determined at STEP 2) and then will be purified (STEP 4) for further use in in vitro tests*

Figure 1.

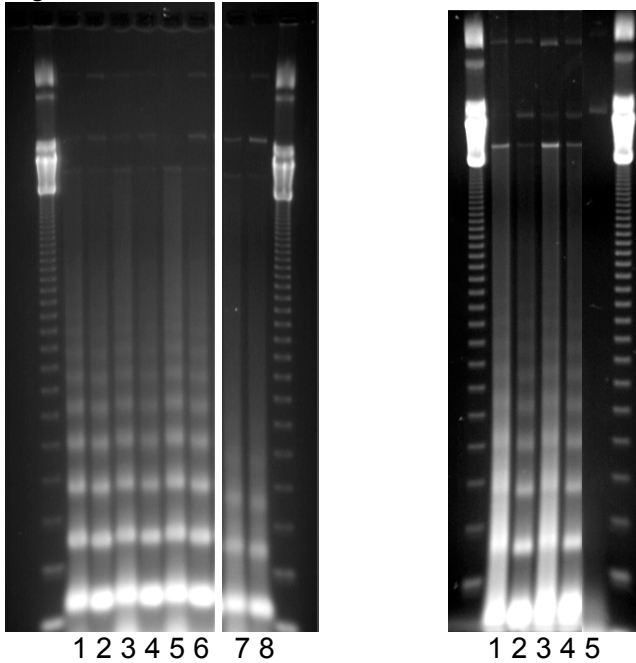


Micrococcal nuclease digestion analysis of the DNA control assembled with histone core and recombinant proteins by the RSF/NAP-1 method. CsCl purified DNA was used in each C.A. tests.

left panel (the MNase provided in the C.A. Kit is used):
 1. 5 mU/digestion (too little enzyme was used)
 2. 50 mU/digestion (**BEST CONDITIONS**)

*middle and right panels:
 MNase other than the one provided in the C..A kit has also been tested:*
 3. 2.5 U /digestion
 4. 10 U /digestion
 5. 0.5 U /digestion
 6. 2.5 U /digestion
 7. 10.0 U /digestion

Figure 2.



C.A. titration followed by digestion analysis.

Micrococcal nuclease digestion analysis of the DNA control assembled with histone core and recombinant by the RSF/NAP-1 method.

C.A. titration is performed using fixed amounts of DNA and a series of histone core amounts, per C.A. reaction. The DNA was purified using the Qiagen kit. Molecular weight marker is located at both sides of the agarose gel.

1. + 2.: low amounts of histone
 3. + 4.: medium amounts of histone core
 5. + 6.: high amounts of histone core
 7. + 8.: -ve control: no RSF, no NAP-1 added.

Right panel: 25.00 mU MNase per digestion (1., 3., 5.)
 50.00 mU MNase per digestion (2., 4., 6.)
 Left panel: 10.00 mU MNase per digestion (1., 3.)
 25.00 mU MNase per digestion (2., 4.)
 mock C.A. (5.)

STEP 3: Final & optimized Chromatin Assembly (with best titer)

After this STEP 3, the obtained assembled chromatin will then be submitted to C.A. purification at STEP 4.

45. Perform a Chromatin Assembly reaction in duplicate with the best condition (determined by titration above). Table 9. below shows an example of chosen condition. See *Points 3. to 7.* above, and repeat these steps working with duplicates (follow: “2 rxns”).

Table 9: Example of optimized chromatin assembly reaction.

C.A. Mix components	C.A. Mix	comments
Buffer A	110.30 μ l	<i>Optimum with your DNA</i>
10 x complete ATP mix	15.00 μ l	<i>Fixed volume</i>
Histones	1.80 μ l	<i>Optimum with your DNA</i>
Blocker	3.00 μ l	<i>Fixed volume</i>
RSF+ NAP-1	3.50 μ l	<i>Fixed volume</i>
Buffer B	6.40 μ l	<i>Fixed volume</i>
Your DNA Sample (2 μ g)	10.00 μ l	<i>Fixed volume</i>
TOTAL VOLUME	150.00 μ l	<i>Fixed volume</i>

46. Incubate at 30°C, overnight.

STEP 4: Assembled chromatin purification

To use clean assembled chromatin in subsequent in vitro experiments is essential. Get rid of free proteins, and change the buffer before proceeding to the in vitro assays with the assembled chromatin. The protocol and results are given below. It is recommended to work in a cold room.

Purify the assembled chromatin.

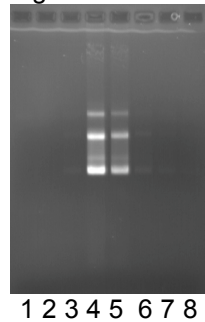
47. Chromatin is purified over a column of 2% or 4% agarose beads (cat#: A-1040-M, ABT, Agarose Bead Technologies: <http://www.abtbeads.com/>)
❖ *Alternatively use a sepharose CL-4B column (Amersham Pharmacia Biotech).*
48. Run the column in a cold room.
49. Pack the resin (4 ml) in a 0.5 x 20 cm glass Econo-column (BioRad).
50. Equilibrate with buffer (10 mM Hepes pH 7.5, 1 mM EDTA).
51. Label 8 new 1.5 ml-tubes (F1, F2, F3, F4, F5, F6, F7 and F8), keep on ice.

52. Mix the 2 C.A. rxns in one tube.
53. Load the chromatin (2 C.A. rxns: total volume of 300 μ l) onto the column bed.
54. Collect the buffer (flow through) in one tube.
55. Allow the sample to sink into the resin completely. That takes 2 to 3 minutes.
56. Load 200 μ l of buffer into the column, do not disturb the column surface.
57. Collect into the first tube (fraction F1).
58. Add 500 μ l of buffer to the top of the column.
59. Collect one more fraction of 500 μ l in the next tube (F2).
60. Then repeat six times the following: Add sequentially buffer aliquots of 250 μ l.
61. Collect 6 fractions of 250 μ l each, in 6 different 1.5 ml tubes (F3 to F8).
62. The chromatin usually elutes from fractions F4-F5, as shown in the Figure 6. below.
63. Wash the column with three column volume of buffer (10 mM Hepes pH 7.5, 1 mM EDTA), and keep at 4°C until next use.

Analyse the profile of the chromatin elution

64. Label new tubes (F1 to F8).
65. For electrophoresis analysis, transfer 20 μ l aliquot from each fraction into the new tubes, add 2.5 μ l of 10% SDS and 5.0 μ l of 6x loading dye.
66. Incubate for 10 minutes at 65°C.
67. Meanwhile, store at 4°C the C.A. rxns, directly use in in vitro assays. Samples can be kept for a week at 4°C.
68. Analyse by eletrophoresis on 1% agarose gel (about 9 cm long).
69. Run at 150 Volts, for about one hour (in our gel conditions).
70. Incubate in Ethidium Bromide-water bath for 30 minutes.
71. Incubate the agarose gel in water for another 30 minutes
72. Under UV light, look at the gel and make a picture (see Figure 6. below).

Figure 6.



Analysis on agarose gel of the purified assembled chromatin fractions, looking at the DNA.

From lane 1 to 8: fraction F1 to F8 respectively

The assembled chromatin is in the fractions F3 and F4.

73. Use the first fraction containing the purified assembled chromatin in in vitro assays.

5. ADDITIONAL PROTOCOLS

- In vitro **Ch**romatin **I**mmunoprecipitation (ChIP) (Pavri et al, 2006).
- In vitro transcription assays (Loyola et al, 2001).
- Electro microscopy (E.M.) (Trojer et al, 2007).

Note: if you wish to perform enzymatic assays. We recommend the salt C.A. method (see Introduction).

Protocols

In vitro ChIP.

- Treat with micrococcal nuclease the purified C.A. samples to generate mononucleosomes.
- Pre-clear the samples by incubating with protein G beads (blocked with salmon sperm DNA).
- Incubate with the antibody of interest overnight at 4°C.
- Recover the immune complexes by adding protein G beads.
- Incubate for one hour.
- Wash the beads.
- Elute the bound DNA.
- Purify the DNA.
- Denature the samples.
- Dot-blot the samples onto a nylon membrane.
- Hybridize with radiolabeled probes.
- Wash the blots, develop, and visualize by autoradiography.

In vitro transcription.

In vitro transcription can be performed using purified assembled chromatin together with the necessary regulators. Basal transcription can be reconstituted using recombinant TBP, TFIIB, TFIIE, TFIIF (expressed in *E. coli*) and highly purified RNA polymerase II and TFIIH (purified from HeLa

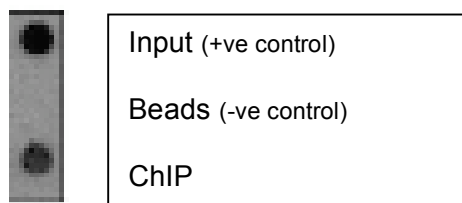
cells). The obtained RNA products are deproteinized, precipitated with ethanol and analysed on 6% polyacrylamide denaturing gel.

Electron microscopy (E.M.).

- Mix purified assembled chromatin with buffer containing spermidine, to a final concentration of 2 mM
- Adsorbe to glow-charged carbon-coated grids
- Wash with a water/graded ethanol series and rotary shadow cast with tungsten
- Examine the samples using a transmission electron microscope.

Results

In vitro ChIP



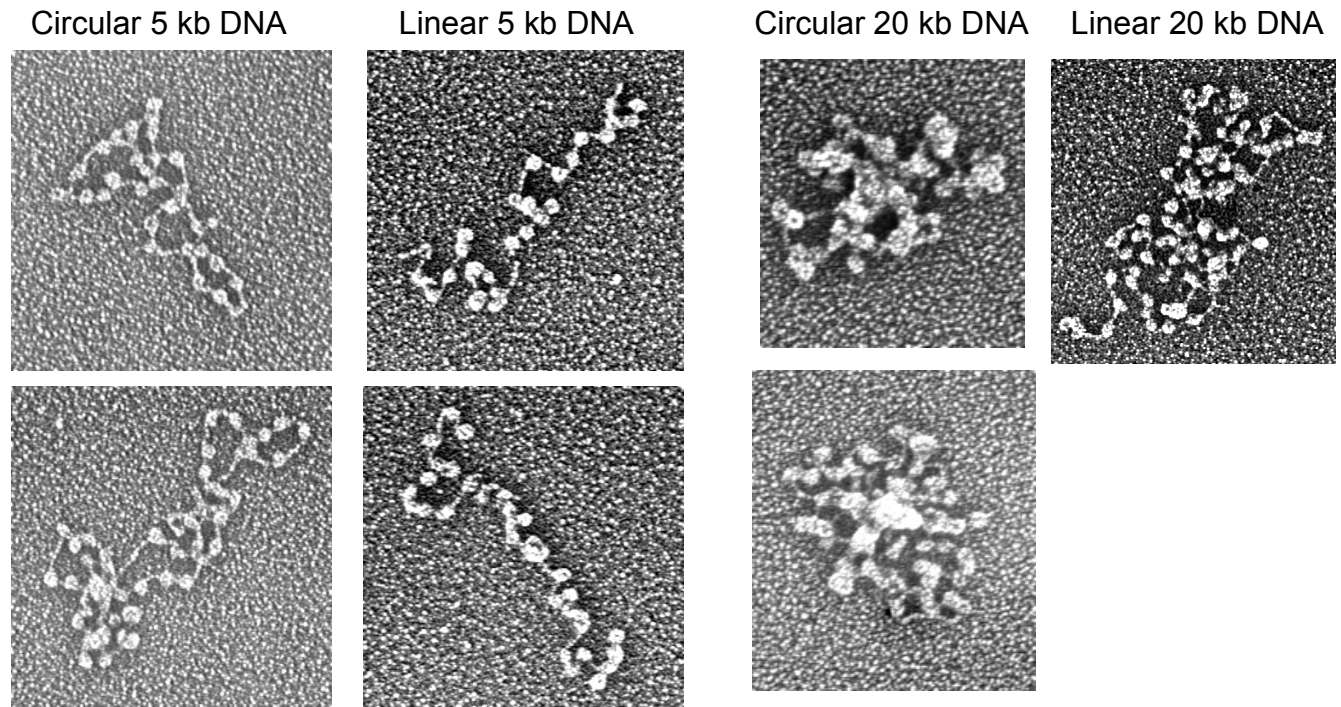
In vitro ChIP results. Assembled chromatin used in transcription assays are subsequently subjected to immunoprecipitation (IP) and dot-blot. Top: input used for the assay (positive control). Middle: beads only, no chromatin used in the IP experiment (negative IP control). Bottom: ChIP.

Transcription assay



Reconstituted transcription on RSF-assembled chromatin is shown above. Transcription was performed using a reconstituted system in the presence (lane 2) or absence (lane 1) of FACT. Products of the transcription reactions were separated by electrophoresis on a denaturing polyacrylamide gel. The full-length transcript in the presence of FACT is clearly seen (lane 2).

Electron microscopy



Electron microscopic visualization of the chromatin assembled by the use of the *in vitro* C.A. kit. As indicated above the pictures, small DNA and large DNA have been used as well as circular and linear DNA. The E.M. experiment was carried out with assembled chromatin using the Diagenode C.A. kit protocol. The protocol for the E.M. is given in the above section. To do E.M. on assembled chromatin in physiological conditions is essential to analyse function and structure of your sample of interest. We can see that the number of nucleosomes is about 25 for the 5kb DNA (left side). Using larger DNA, the nucleosomes are still visible, but the assembled chromatin is forming clumps and very compact structure is obtained mostly with circular DNA (right side).

6. TROUBLESHOOTING GUIDE

Critical steps	Trouble	Solution
Preparation of the DNA	Not good quality Give a smear or less than 6 bands when you MNase digest	CsCl preparation of DNA is best See our protocol in this manual
C.A. titration	If not done, then the C.A. reaction might fail	Histone:DNA ratio is very important
	Too little histones Band lower than mononucleosome	Titration is essential as the Histone/DNA ratio is very important (see our protocol)
	Too much Histones Lot of DNA can not be cut Or smear	Titration is essential as the Histone/DNA ratio is very important (see our protocol)
MNase digestion	If you see less than 6 clear bands: the sample is overdigested	Digest with less enzyme.
	If you get a smear but can see some bands (vaguely): the sample is underdigested	Digest with more enzyme.
10x ATP Mix	It could stop working	Make it fresh every single time
Proteins to aliquot and keep at -80°C	It could stop working	Do not freeze-thaw more than two to three times
DNA sample	Using too little DNA or too much DNA will give you bad C.A.	Use the DNA sample at the right concentration: use 2 ug per C.A. reaction. Use our DNA control as a reference when measuring your DNA sample concentration.
Buffer C	The Stop MNase buffer can show some precipitates	Warm it up before use
Tubes	Mainly 1.5 ml tubes, no need of siliconized tubes	Our results: no siliconized tubes are used
MNase digestion analysis	Bad image	Running of the agarose gel Do not add Eth Bromide into the gel Stain the gel after migration
C.A. purification	Chromatin dissociates	Keep at 4°C
	Bad results might be obtained in in vitro	Purification is essential. A purified assembled chromatin will give your better

	tests if purification is not performed	results.
C.A. storage	Bad results might be obtained in in vitro tests if purification is not performed soon enough after C.A. reaction.	Do not keep the unpurified C.A. for more than 1 day
	Use the purified assembled chromatin shortly after purification and store at the right temperature.	No longer than one week at 4°C Do not freeze
Work with unpurified C.A.	Results ca not be convincing if you work with unpurified assembled chromatin in your in vitro tests	Get rid of proteins first by purification. The purification step is essential.
Combination of C.A. reactions for one purification	To get more material after purification, combine 2 C.A.	It is advised to combine two C.A. reactions because one reaction is a little dilute to run a column. So, it is better to run a column by combination of 2 C.A. reactions.
Other histones	Use of histones that are not provided in the kit Recombinant histone can be used (native is provided)	Possible, but titration is essential The use of recombinant histones is slightly less efficient in C.A. Pre-acetylation recombinant histones is advised (Loyola, 2001) Note that the histones given in the kit are at the concentration of 0.9 µg /µl
Centrifugations	x g and rpm	$g = 11.18 \times r \times (\text{rpm}/1000)^2$; knowing that r is the radius of rotation in mm. (http://www.msu.edu/~venkata1/gforce.htm).

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8. ORDERING INFORMATION

Diagenode s.a. Europe, Asia & Australia

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4000 Sart-Tilman Liège BELGIUM
Phone: +32 (0) 4 364 20 50 and Fax: +32 (0) 4 364 20 51
Email: Info@diagenode.com

Diagenode Inc. USA

376 Lafayette Road, Suite 202
Sparta, NJ 07871
Phone : +1 973 300 0976
Fax: +1 973 300 1862 +1
Email: infousa@diagenode.com

Diagenode website: <http://www.diagenode.com/>

9. DIAGENODE RELATED PRODUCTS

Chromatin Assembly kit:

- ❖ **C.A. kit™:** catalog # ca-vitro-001
- ❖ **DNA module for C.A.:** catalog # ca-vitro-002

Chromatin and DNA shearing

- ❖ **Bioruptor™:** catalog #: UCD-200

Advantages of the Bioruptor™ - over the other sonicator apparatus -:

- With the Bioruptor™ the quality of the sheared chromatin (and DNA) is greater: a compact pack of chromatin (and DNA) fragments of the right size is obtained rather than a smear. In the chromatin (and DNA) sheared using the Bioruptor™, the amount of different fragment sizes is reduced and the presence of too long fragments is also eliminated, these being a source of background in the subsequent ChIP (and Methyl DNA IP) experiments. Thus, the high quality of the sheared chromatin (and DNA) obtained with the Bioruptor™ is essential to get better results in ChIP (and Methyl DNA IP), by decreasing dramatically the background noise. The way to test the shearing efficiency is to look at the extent of DNA fragmentation using agarose gel electrophoresis.
- With chromatin (and DNA) sheared with the Bioruptor™, the reproducibility is close to 100% (other sonicator apparatus gives good shearing in no more than 1 out of 5 trials)
- Note that the Bioruptor™ can also be used for many other applications.

- ❖ **ChIP Sonication Ctl kit™:** catalog #: kch-sonctl-001
- ❖ **Shearing-ChIP kit™:** catalog #: kch-shchro-040
- ❖ **Shearing module:** catalog #: kch-redmod-100
- ❖ **Large Shearing module:** catalog #: kch-redmod-400

Chromatin IP kits

- ❖ **Red ChIP kit™ (TF-ChIP):** catalog #: kch-redTBP-012
- ❖ **Orange ChIP kit™ (Histone ChIP):** catalog #: kch-orgHIS-012
- ❖ **OneDay ChIP kit™ (Rapid ChIP):** cat #: kch-oneDIP-060,-180
- ❖ **LowCell# ChIP kit™ (Magnetic ChIP):** catalog #: kch-magfew-016

ChIP grade Reagents, separately available

- ❖ Protease Inhibitor Mix (20 µl): catalog #: kch-107-020
- ❖ Protease Inhibitor Mix (100 µl): catalog #: kch-502-100
- ❖ Protease Inhibitor Mix (300 µl): catalog #: kch-502-300
- ❖ Protease Inhibitor Mix (1 tablet): catalog #: kch-207-001
- ❖ Protease Inhibitor Mix (1 tablet): catalog #: kch-405-001
- ❖ Protease Inhibitor Mix (1 large tablet): catalog #: kch-306-001
- ❖ DNA co-precipitant (100 µl): catalog #: kch-216-100
- ❖ DNA precipitant (1000 µl): catalog #: kch-217-001
- ❖ Proteinase K (100 µl): catalog #: kch-507-100

- ❖ Proteinase K (250 µl): catalog #: kch-507-250
- ❖ Sterile Water (28 ml): catalog #: kch-508-028
- ❖ Sterile Water (85 ml): catalog #: kch-508-085
- ❖ GenDNA RNase (10 µl): catalog #: mcg-605-010
- ❖ 1 M Sodium butyrate (1 ml): catalog #: kch-817-001
- ❖ ProtG-coated paramagnetic beads: catalog #: kch-818-220
- ❖ Negative Ctrl IgG from mouse: catalog #: kch-819-015
- ❖ PBS (50 ml) : catalog #: kch-820-050

- ❖ Blocked PA/G beads (630 µl) : catalog #: kch-208-630
- ❖ pre-Blocked PA beads (880 µl) : catalog #: kch-406-880
- ❖ unblocked Protein A beads (8 ml): catalog #: kch-503-008
- ❖ unblocked Protein A beads (2.8 ml): catalog #: kch-503-028
- ❖ unblocked Protein A beads (880 µl): catalog #: kch-503-880

- ❖ Magnetic Rack (16 rxns): catalog #: kch-816-001

Some of our ChIP grade antibody directed against:

- TBP: catalog #: MAb-002-100
- Histone H3 [K4me3]: catalog #: pAb-003-024
- Histone H3 [K9ac]: catalog #: pAb-004-044
- Histone H3 [K9/14ac]: catalog #: pAb-005-044
- Histone H3 [K9me3]: catalog #: pAb-056-050
- Histone H3 [K9me3]: catalog #: CS-056-050
- Histone H4 [K20me3]: catalog #: pAb-057-050
- NF-YB: catalog #: pAb-007-100
- p63: catalog #: pAb-008-050
- ERalpha: catalog #: MAb-009-050
- GR : catalog #: MAb-010-050
- Ty1-tag : catalog #: AC-055-100
- HDAC1 : catalog #: pAb-053-050
- Pol II (RNA polymerase II): catalog #: MAb-054-050
- Plus many other antibodies: visit our website: <http://www.diagenode.com/>

Peptides

visit our website: <http://www.diagenode.com/>

Primer pairs

visit our website: <http://www.diagenode.com/>

Methylated DNA IP

- ❖ **Complete METHYL kit™:**
- ❖ **Methyl DNA IP module:**
- ❖ **GenDNA and qPCR modules:**
- ❖ **meDNA IP blocked ProtA/G beads (300 µl):**

catalog #: mc-green-003

catalog #: mc-green-001

catalog #: mc-green-002

catalog #: mcg-606-300

Methyl DNA IP grade antibody directed against:

- 5-methyl Cytidine:

catalog #: MAb-5MECYT-100,-200,-500

DNA Methylation Detection kits™

- 25 rxns:

catalog #: MEA-BISLPH-025

- 96 wells / vacuum:

catalog #: MEA-BISWEV-096v

- 96 wells / centrifugation:

catalog #: MEA-BISWEC-096c

Methyl Kit for methylated DNA immunoprecipitation (Methyl DNA IP)

Include in your IP the provided controls to directly estimate the methylation status of your DNA sample.

Kit designed to perform specific DNA methylation analysis and internal IP quality control.

- ❖ GenDNA module included for the preparation of DNA.
- ❖ Optimized protocol for DNA shearing.
- ❖ Brand new method: methylated DNA and unmethylated DNA internal IP controls are provided to be used together with your DNA sample - "All in one Tube" - allowing direct correlation between IP'd material and methylation status.
- ❖ One format: 10 IPs per kit.

RED ChIP Kit: The Transcription Factor-ChIP

Work with our straightforward and optimized ChIP method.

Kit designed to perform ChIP assays on transcription factors.

- ❖ Traditional method: 3 days from cell collection to PCR results (2 O.N. incubations).
- ❖ Includes a chromatin shearing module and a qPCR module.
- ❖ TBP: Control antibody.
- ❖ One Format: 18 IPs per kit.

ORANGE ChIP Kit: The Histone-ChIP

Work with our straightforward and optimized ChIP method.

Kit designed to perform ChIP assays on Histones and Histone Modifications.

- ❖ Simplified protocol.
- ❖ Traditional method: 3 days from cell collection to PCR results (2 O.N. incubations).
- ❖ Includes a chromatin shearing module and a qPCR module.
- ❖ Histone H3K4me3: Control antibody.
- ❖ One format: 18 IPs per kit.

OneDay ChIP Kit: The Rapid ChIP

Analyse many samples in a short period of time.

Kit designed to perform ChIP assays in abundance.

- ❖ ChIP in 2 hours.
- ❖ Recover the DNA ready-to-PCR in 2 hours.
- ❖ New method: one day from cell collection to PCR results.
- ❖ Two formats: 60 IPs or 180 IPs per kit.

LowCell# ChIP kit: The Magnetic ChIP

Work with a low number of cells per ChIP.

Kit designed to perform ChIP assays using magnetic IP and small volumes of reagents.

- ❖ Validated with low number of cells for histone- and TF-ChIP assays.
- ❖ Use chromatin from 10,000 cells or less to perform histone ChIPs.
- ❖ Ideal to test one sample with 6 to 14 antibody to modified histones or transcription factors.
- ❖ Magnetic rack available at Diagenode. Ensures the best ChIP conditions by providing cooling at all times to avoid degradation.
- ❖ Includes buffers for chromatin shearing and qPCR primer pairs.
- ❖ One Format: 16 IPs per kit.

In vitro Chromatin Assembly Kit (C.A.)

Use in your C.A. experiments, the provided kit DNA control and proteins.

Kit designed to perform in vitro Chromatin Assembly Optimization and subsequent Purification.

- ❖ Brand new method: Perform the C.A. step-by-step starting with a titration and C.A. analysis. Then, purify the C.A. before any in vitro applications.
- ❖ Optimized protocol for DNA preparation prior to C.A.. (module included)
- ❖ C.A. tested in a series of in vitro applications: protocols included.
- ❖ One format: 20 rxns per kit, using 2 µg of DNA per assay.

10. C.A. KIT: catalog # of each component

cat #: ca-vitro-001

C.A. Kit	cat#	20 C.A./one KIT	Storage
Histones	ca-701-075	75 μ l	-80°C
Blocker	ca-702-120	120 μ l	-20°C
RSF +NAP1	ca-703-090	90 μ l	-80°C
Buffer A	ca-704-006	6 ml	-20°C
Buffer B	ca-705-500	500 μ l	RT
10x ATP Mix-1	ca-706-330	330 μ l	-20°C
10x ATP Mix-2	ca-707-060	60 μ l	-20°C
10x ATP Mix-3	ca-708-040	40 μ l	-80°C
DNA Control	ca-709-060	60 μ l	-20°C
MNase	ca-710-030	30 μ l	-80°C
Buffer C	ca-711-150	150 μ l	RT
Proteinase K	ca-712-030	30 μ l	-20°C
Buffer D	ca-713-006	6 ml	RT
Co-precipitant	ca-714-075	75 μ l	-20°C
Precipitant	ca-715-600	600 μ l	RT

cat #: ca-vitro-002

DNA MODULE for C.A.			
Buffer 1.	ca-801-050	50 ml	4°C
Buffer 2.	ca-802-150	150 ml	RT
Buffer 3.	ca-803-100	100 ml	RT