Chromatin assembly kit

Product	Format	Catlog no.
Chromatin Assembly Kit	20 reactions	EPX-01-CAK

Each Chromatin Assembly Kit contains reagents for 20 reactions. A reaction is defined as chromatin generated from 1 µg of DNA.

Background:

In eukaryotic cells DNA is associated with histones to form chromatin. The basic unit of chromatin is the nucleosome consisting of 146 bp of DNA wrapped around an octamer of the four core histones H2A, H2B, H3 and H4. Chromatin assembly is a fundamental process that is tightly linked to DNA replication and enables the cell to faithfully duplicate the chromosomes. Chromatin assembly can also occur independently of replication to turn-over histones, for instance during transcription or DNA damage repair. The chromatin-remodeling factor ACF (Acf1/ISWI complex) cooperates with the histone chaperone NAP-1 to assemble, in vitro, a regularly spaced chromatin in an ATP-dependent manner.

Chromatin assembly kit applications:

The Chromatin Assembly Kit allows you to assemble chromatin on you favorit DNA sequence. The kit generates assembled chromatin in less than 6 hours with few manipulations, providing you with material that is ideal for downstream applications such as *in vitro* transcription, recombination, replication assays, histone modifications analysis and in vitro ChIP assays. The kit contains recombinant human histones, the recombinant histone chaperone NAP-1 and the recombinant Acf1/ISWI complex, which catalyzes the deposition of histones into a regulary spaced nucleosome arrays in an ATP-dependent manner.

Kit Storage:

The chromatin assembly kit should be stored at -80°C. Avoid multiple freeze-thaw cycles.

Guarantee:

For research use only. Each lot has been evaluated by SDS-PAGE and tested for chromatin assembly. Products guaranteed stable for six month from date of receipt when stored properly.

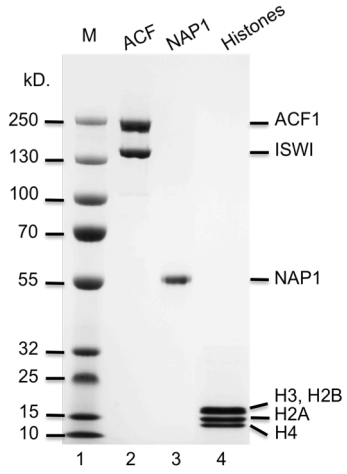

Figure 1. SDS-PAGE gel of recombinant nucleosome assembly assay components.

Kit Components and recommendations:

The following components should be thawed, gently mixed and aliquoted as described below.

Recombinant NAP-1 Protein

Prepare 10 aliquots of 5 µl each. Store at -80°C. This component should only go through 3 freeze-thaw cycles.

Recombinant ACF Complex

Prepare 10 aliquots of 5 µl each. Store at -80°C. This component should only go through 2 freeze-thaw cycles.

Human recombinant Core Histones

Prepare 10 aliquots of 1.5 µl each. Store at -80°C. This component may go through 5 freeze-thaw cycles.

0.1M ATP

This component should be aliquoted and used fresh each time. We recommend that you make 10 aliquots of 5 µl each. Store all aliquots at -80°C.

Reagent	Quantity	Storage/stability
Recombinant NAP-1 Protein (2mg/ml)	50µl	-80°C for 12 months
Recombinant human core histones (2mg/ml)	15µl	-80°C for 12 months
Recombinant ACF complex (0.2mg/ml)	50µl	-80°C for 6 months
CAB (Chromatin assembly buffer) 10X	1000µl	-80°C for 12 months
0.1M ATP	20µl	-80°C for 12 months
Supercoiled DNA (1 mg/ml)	20µl	-20°C for 12 months
MNase Buffer 2X	1000µl	-20°C for 12 months
Micrcoccal Nuclease (0.05U/µl)	40µl	-20°C for 12 months
0.1 M EGTA	100µl	-20°C for 12 months

Additional materials required:

- Water bath
- Sterile distilled water (dH2O)
- Microcentrifuge Tubes
- 10% SDS
- phenol/chloroform
- chloroform-Isoamyl-alcohol (24 :1 V/V)
- 5M NH4OAc
- 100% and 70% ethanol
- TE (10 mM Tris, 1 mM EDTA pH 7.8)
- Reagents for purifying high-quality supercoiled DNA (*e.g.* Qiagen Plasmid Maxi Kit, Catalog No. 12163).
- 100 bp DNA ladder (*e.g.* Invitrogen, Catalog No.15628-019)
- Standard Tris/Glycine agarose gel supplies 5X Tris Glycine Buffer: 26.75 g Tris base; 144 g Glycine, 1.36 g EDTA and dH20 to 1L.

Chromatin assembly protocol:

Prior to starting the assay please prepare the following:

A. Preparation of Sample DNA

Prepare high quality DNA at 1 µg/µl. The quality of the DNA is important for the efficiency of the chromatin assembly reaction. The quality of the DNA should be checked on agarose gel to ensure that the DNA is not contaminated with RNA. In general, high-quality DNA can be prepared by standard CsCl gradient methods or a commercial kit may be used *e.g.* Qiagen Plasmid Maxi Kit, Catalog No. 12163.

B. Setting the Chromatin assembly reaction

1. Set water bath to 30°C.
2. Add the following reagents in the order shown in the table below.

Note: Keep reaction reagents on ice during reaction set-up and return to appropriate -80°C or -20°C storage immediately after use.

Component	Sample	Positive control	Negative control
dH2O	15µl	15µl	20.75µl
Chromatin Assembly buffer 10X	2.5µl	2.5µl	2.5µl
Recombinant Core Histones	0.75µl	0.75µl	-
Recombinant NAP-1	2.5µl	2.5µl	-

3. Gently vortex the samples. Centrifuge to collect material at the bottom of the microcentrifuge tube. Incubate on ice for 15 minutes.

4. Add the following reagents in the order outlined in the table below.

Component	Sample	Positive control	Negative control
Supercoiled DNA (Control)	-	1µl	1µl
Sample DNA	1µl (1µg)	-	-
0.1M ATP	0.75µl	0.75µl	0.75µl
Recombinant ACF complex	2.5µl	2.5µl	-
Total Volume	25µl	25µl	25µl

5. Gently mix the samples and spin down at low speed (500 x g, 3000rpm) to collect material at the bottom of the microcentrifuge tube. Incubate at 30°C for 3 hours. After incubation, the sample may be stored at 4°C for up to 3 days. Chromatin should not be frozen at this point. The assembled chromatin can be used directly in downstream assays.

Note: This protocol has been optimized for supercoiled plasmid DNA but it will also work for linear DNA.

C. Analysis of Assembled Chromatin by Partial Digestion

The **efficiency of the chromatin assembly** reaction can be evaluated by partial micrococcal nuclease (MNase) digestion (see Figure 2). Partial digestion with the micrococcal nuclease (MNase) will reveal periodic spacing of assembled nucleosomes. After the chromatin is digested, it is deproteinated. The resulting DNA fragments are resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. High-quality chromatin should yield 7 or more distinct bands (see Figure 2).

1. Add 1 volume of MNase Buffer to the assembled chromatin reaction.
2. Mix contents of the tube gently and incubate at 37°C for 5 min.
3. Digest each reaction with 2 µl of MNase for 10 minutes at 37°C.

Note: We recommend that two time points be tested for each enzymatic digestion *e.g.*, split the 50 µl reaction into two tubes and digest each aliquot with 1 µl of MNase for 5 and 10 minutes respectively. Splitting the reaction below 25 µl will make reaction efficiency difficult to visualize by agarose gel electrophoresis.

4. Add 2 µl of 0.1M EGTA (4mM final concentration) to stop the MNase reaction. Mix well. Incubate on ice for 5 min. The reactions may be stored at -20°C at this point.

Note: The quantity of enzyme and time of incubation may need to be optimized for the sample DNA. The conditions in this protocol are optimized for the included positive control DNA, which is supercoiled circular DNA.

D. Deproteinization

1. Add 10% SDS to a final concentration of 0.5% (2.5 µl per 50 µl or 1.25 µl per half volume reaction).
2. Add 1 µl (or 0.5 µl per half volume reaction) of Proteinase K.
3. Incubate at 55°C for 30 minutes.
4. Add sterile dH2O to final volume of 200 µl.
5. Perform a phenol/chloroform extraction once, *i.e.* add 200 µl of phenol/chlorofom, vortex samples and spin for 5 minutes at room temperature.
6. Remove the top 200 µl (supernatant)
7. Add 1 µl Glycogen, 200 µl of 5 M NH4OAc and 1 ml 100% EtOH.
8. Incubate at -20 for 30 minutes
9. Spin for 15 minutes at 4°C. Discard the supernatant. (A little white pellet is visible at this point. Take care not to disturb the pellet, leave 10-20 µl close to the pellet).
10. Wash DNA pellet by adding 1 ml 70% EtOH, and centrifuge at 4°C for 5 minutes. Remove as much of the EtOH as possible.
11. Air dry for approximately 5 minutes.
12. Resuspend pellet in 8 µl dH2O (or TE). Add 2 µl 5X Orange G dye.

    Note: The Orange G dye can be replaced by another dye. However, some dyes *e.g.* Bromophenol Blue migrate with bands at 100 bp.

13. The sample reactions can be stored at -20°C or analyzed immediately by agarose gel electrophoresis.

E. Agarose Gel Electrophoresis

1. Perform agarose gel electrophoresis (1.5% agarose gel in cold 1X Tris-Glycine buffer in a 4°C cold room). Run the samples and a 100 bp ladder at 80 V for approximately 1 hour and 30 minutes. Add ethidium bromide to the gel and the buffer. Terminate the electrophoresis when the DNA dye front reaches the desired position of the gel.

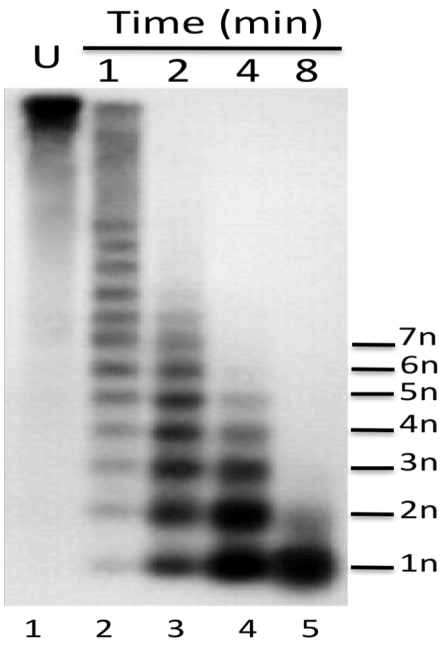

Figure 2.

MNase digestion kinetic of chromatin assembled on supercoiled DNA using the Chromatin Assembly Kit protocol. Regulary spaced nucleosomes are visualized on agarose gel and labeled from 1 to 7n.

Lane 1: Undigested DNA ; Lane 2: Circular DNA digested with MNase for 1 minute; Lane 3: Circular DNA digested with MNase for 2 minutes; Lane 4: Circular DNA digested with MNase for 4 minutes; Lane 5: Circular DNA digested with MNase for 8 minutes.

Appendix

Troubleshooting guide

PROBLEM	POSSIBLE CAUSE	RECOMMENDATION

Less than 6 nucleosome bands on agarose gel without a smear	The chromatin was overdigested	Reduce the MNase Concentration.
	The histone/DNA ratio is not good.	Use less DNA or more core histones
	Sample lost during purification	Check carefully the DNA precipitation step and add more glycogen.
Less than 6 nucleosome bands on agarose gel with a smear	The chromatin was under digested	Use more MNase or increase the digestion time.
	The histone/DNA ratio is not good.	Use more DNA or less core histone
	Low efficiency of the assembly	Poor quality DNA. The ACF complex is not working