

## In vitro Chromatin Assembly Kit (Cat No. HA-001)

### I. Reagents\*

- A. Control DNA template (pNAS, 2 µg, 0.1 mg/ml).
- B. pNAS primer mix (40 µl, 100 pmol/µl).
- C. 10x Dilution Buffer (200 µl): 0.2 M Hepes-NaOH, pH 7.4, 0.1 mM EDTA, 0.5 mM PMSF, and 10 mM DTT.
- D. 5 M NaCl (200 µl).
- E. HeLa core histones (100 µg, 2 mg/ml in 2.5 M NaCl)
- F. 1x Dilution Buffer (0.5 ml): 20 mM Hepes-NaOH, pH 7.4, 0.1 mM EDTA, 0.5 mM PMSF, and 1 mM DTT
- G. 2x Storage Buffer (1 ml): 40 mM Hepes-NaOH, pH 7.4, 0.2 mM EDTA, 0.5 mM PMSF, 2 mM DTT, 0.1% NP-40, 0.6 mg/ml BSA, and 30% glycerol.

*\*All reagents should be completely thawed, mixed by vortex, and briefly centrifuged. They should be always kept on ice during the preparation of reaction mixture described below and returned to -70°C freezer immediately after use.*

### II. In vitro Nucleosome Assembly<sup>a</sup>

#### A. Preparation of internal control 152 bp dsDNA (Xenopus 5S rDNA)<sup>b</sup>.

**Step 1.** Prepare PCR reaction mix (100 µl) as follows:

<b>Reagent A</b>	1 µl
<b>Reagent B</b>	2 µl
dNTP mix <sup>c</sup>	N µl (to 0.25 mM)
α- <sup>32</sup> P-dCTP <sup>c</sup>	10 µl
10x PCR buffer <sup>c</sup>	10 µl
Taq polymerase <sup>c</sup>	5 units
Deionized H <sub>2</sub> O <sup>c</sup>	to a final volume of 100 µl

**Step 2.** Perform PCR reaction:

95°C-30 sec	1 cycle
95°C-15 sec	} 35 cycles
55°C-30 sec	
72°C-1 min	
72°C/7 min	1 cycle

**Step 3.** Purify PCR product using spin-column<sup>c</sup>.

**Step 4.** Measure the radioactivity with 1 µl aliquot.

**Step 5.** Estimate the DNA concentration as follows:

DNA concentration (pmol/µl or 100 ng/µl) = CPM measured/77440 CPM.

### B. Chromatin assembly reaction

**Step 1.** Prepare reaction mix (20 µl/reaction) in the following order and ratio.

<b>Reagent C</b>	2 µl
<b>Reagent D</b>	0-3.25 µl (see below)*
Substrate DNA <sup>c</sup>	2-8 µg (see below)*
<sup>32</sup> P-labeled control	1 µl
152 bp DNA (see IIA)	
<b>Reagent E</b>	1.5-8 µl (see below)*
Deionized H <sub>2</sub> O <sup>c</sup>	to a final volume of 20 µl.

\*For efficient nucleosome formation, DNA, core histones, and 5 M NaCl should be mixed in the following ratio. More importantly, core histones should be lastly added to the mixture.

#### 1. Linear DNA

DNA (µg)	Core histones	5 M NaCl
2	1.5 µl	3.25 µl
4	3 µl	2.5 µl
6	4.5 µl	1.75 µl
8	6 µl	1 µl

#### 2. Circular DNA

DNA (µg)	Core histones	5 M NaCl
2	2 µl	3 µl
4	4 µl	2 µl
6	6 µl	1 µl
8	8 µl	0

**Step 2.** Incubate at 37°C for 30 min.

**Step 3.** Serially dilute the reaction mix with **Reagent F** and at each dilution, incubate the mixture at 30°C for 20 min.

- i. 4 µl
- ii. 7 µl
- iii. 12 µl
- iv. 25 µl

**Step 4.** Add 66 µl of **Reagent G** and 66 µl of deionized H<sub>2</sub>O<sup>c</sup>.

**Step 5.** Incubate at 30°C for 30 min, followed by overnight incubation on ice or at 30°C.

**Step 6.** Analyze 10 µl aliquots of assembled chromatin on a native gel (5% polyacrylamide gel).

**Step 7.** Store the assembled chromatin below -20°C before use.

- a. *Science* 265, 53-60 (1994).
- b. *FEBS Letters* 523, 7-11 (2003).
- c. Not provided.