EpiMark® Nucleosome Assembly Kit

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E5350S 004140415041

E5350S

20 reactions **Lot: 0041404**

RECOMBINANT Store at –20°C **Exp: 4/15**

Description: The EpiMark Nucleosome Assembly Kit is used to make unmodified recombinant human nucleosomes with user-supplied DNA or the provided control DNA. Purified recombinant human Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer are mixed with DNA at 2 M NaCl. To generate nucleosomes, the salt concentration is lowered by dilution or dialysis allowing each histone tetramer to associate with two histone dimers and form the histone octamer on the DNA (1,2). A method for assaying nucleosome formation by gel shift assay is included. These nucleosomes may serve as a better substrate for enzymes that are inactive on the DNA or one of the core histones alone (3,4). Each reaction creates nucleosomes from ~1 µg of 208 bp DNA and may be scaled depending on the experiment.

Applications:

- ChIP assay
- HAT assay
- Enzymatic modification assay, (e.g., methylation)

Advantages:

- Highly pure, recombinant system
- Components stable for one year
- Pre-formed histone dimer and tetramer complexes simplify octamer formation
- Dilution protocol only requires a few hours for assembly

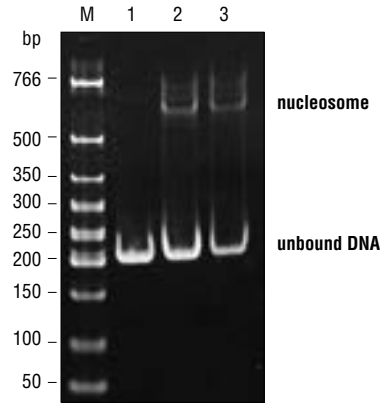
Kit Components:

20 µM Histone H2A/H2B Dimer	100 µl
10 µM Histone H3.1/H4 Tetramer	100 µl
10 µM Nucleosome Control DNA	20 µl

Quality Control

The EpiMark Nucleosome Assembly Kit has been tested using the control DNA provided as described in the Dilution Assembly Protocol.

The control reaction yields a characteristic shift on 6% native polyacrylamide gel from 208 bp to ~700 bp.



Nucleosome formation. *A gel shift assay allows visualization of complex formation when various ratios of Octamer* to DNA are used to form mononucleosomes using the provided nucleosome control DNA. When assembly reactions are run on 6% polyacrylamide gel in 0.5X TBE, a characteristic shift occurs from 208 bp to approximately 700 bp. M: Low Molecular Weight DNA Ladder (NEB #N3233) Lane 1: Nucleosome Control DNA, Lane 2: 0.5:1 ratio of Octamer* to DNA, Lane 3: 1:1 ratio of Octamer* to DNA.*

**Octamer = 2:1 mix of Histone H2A/H2B Dimer and Histone H3.1/H4 Tetramer.*

General Guidelines

Molar Ratio
(2 Dimers : 1 Tetramer : 1 DNA Binding Site)

Because the correct ratio of DNA to protein determines the efficiency of an assembly, the kit components have been formulated to have equal volumes of each added in a reaction. After assembly, the reactions should contain 5–10% unbound DNA to mitigate aggregate formation which occurs when protein is in excess.

DNA Substrate

The Nucleosome Control DNA (208 bp) contains one possible binding site for an octamer. This enables visualization by gel shift assay due to the binding of the octamer and a characteristic shift from 208 bp to ~700 bp on a 6% native polyacylamide gel. When using user-supplied substrate DNA, optimization may be required to determine the number of possible binding sites per molecule. A starting point for the amount of user supplied DNA to add per reaction can be determined using the formulas described in the DNA concentration formula section. Then, keeping the amount of DNA constant, the amount of octamer can be varied to find an optimal ratio of DNA to protein. To conserve DNA and protein during optimization, we would recommend the dilution protocol for 25 pmol.

Other Considerations

- Because stability can be an issue for the octamer *in vitro* without DNA, we provide the Dimer and Tetramer separately.
- Set up each reaction with dH_2O, 5 M NaCl, DNA and protein such that the final concentration is 2 M NaCl, noting that the Dimer and Tetramer are supplied in 2 M NaCl containing buffer.
- Always add the Dimer and Tetramer last!
- Because nucleosomes can dissociate when too dilute, it is recommended to keep the final protein concentration above 10 µg/ml (5).

Protocols

Two different protocols are available. The quickest is the dilution assembly protocol with assembly being ready in less than three hours. It is ideal for optimization since smaller volumes of all components may be used. The dialysis protocol does take longer but the concentration differences from starting material are minimized and it requires less sample handling.

Dilution Assembly Protocol [5]

Reactions can be scaled up or down depending on the final nucleosome requirement. Methods for 50 pmol and 25 pmol are presented. If the scale is adjusted, it is important to also adjust 5 M NaCl addition such that the starting concentration in the reaction is 2 M NaCl. With sequential dilutions and incubation, the salt concentration is lowered to 0.25 M NaCl, allowing the octamer to bind the DNA and form the nucleosome core particle.

Materials Required but not Supplied

5 M NaCl

Dilution Buffer: 10 mM Tris, pH 8.0

6% Polyacrylamide gel with gel apparatus and gel buffer (ex: Invitrogen 6% DNA retardation gel)

100% Glycerol

TriDye™ 100 bp DNA Ladder (NEB #N3271)

1X TBE

For 50 pmol

This reaction, as described, can yield a maximum of 50 pmol nucleosome in 160 µl (0.3 pmol/µl; 0.3 µM nucleosome; 33.7 µg/ml protein).

1. Place 200 µl of Dilution buffer per reaction at room-temperature.
2. Prepare the Reaction Assembly Mix on ice in the following order (for user-supplied substrate, suggested ratios have been included):

		For Optimizing User-supplied DNA Substrate		
	Control DNA only (50 pmol)	**0.5 to 1 Octamer to DNA**	**1 to 1 Octamer to DNA**	**1.5 to 1 Octamer to DNA**
Water	1 µl	0 to 4.5 µl	0 to 7 µl	0 to 1.5 µl
5M NaCl	4 µl	6 µl	4 µl	2 µl
DNA	5 µl (10 µM)	50 pmol	50 pmol	50 pmol
20 µM Dimer	5 µl	2.5 µl	5 µl	7.5 µl
10 µM Tetramer	5 µl	2.5 µl	5 µl	7.5 µl
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**

The Dimer and Tetramer are supplied in 2 M NaCl.

3. Incubate reactions at room temperature for 30 minutes.
4. Add 7 µl room temperature dilution buffer to each reaction. This brings the reactions to 1.48 M NaCl, 27 µl total volume. Incubate at room temperature for 30 minutes.
5. Add 13 µl room temperature Dilution Buffer to each reaction. This brings the reactions to 1.0 M NaCl, 40 µl total volume. Incubate at room temperature for 30 minutes.
6. Add 27 µl room temperature Dilution Buffer to each reaction. This brings the reactions to 0.6 M NaCl, 67 µl total volume. Incubate at room temperature for 30 minutes.
7. Add 93 µl room temperature Dilution Buffer to each reaction. This brings the reactions to 0.25 M NaCl, 160 µl total volume. Incubate at room temperature for 30 minutes.
8. Store samples at 4°C.
9. Use gel shift assay to analyze samples.

(see other side)

For 25 pmol

This reaction can yield a maximum of 25 pmol nucleosome in 80 µl (0.3 pmol/µl; 0.3 µM nucleosome; 33.7 µg/ml protein).

1. Place 100 µl of Dilution Buffer per reaction at room temperature.
2. Prepare the Reaction Assembly Mix on ice in the following order (for user-supplied substrate, suggested ratios have been included):

		For Optimizing User-supplied DNA Substrate		
	Control DNA only (25 pmol)	**0.5 to 1 Octamer to DNA**	**1 to 1 Octamer to DNA**	**1.5 to 1 Octamer to DNA**
Water	0.5 µl	0 to 4.5 µl	0 to 7 µl	0 to 1.5 µl
5M NaCl	2 µl	3 µl	2 µl	1 µl
DNA	2.5 µl (10 µM)	25 pmol	25 pmol	25 pmol
20 µM Dimer	2.5 µl	1.25 µl	2.5 µl	3.75 µl
10 µM Tetramer	2.5 µl	1.25 µl	2.5 µl	3.75 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

The Dimer and Tetramer are supplied in 2 M NaCl.

3. Incubate reactions at room temperature for 30 minutes.
4. Add 3.5 µl room temperature dilution buffer to each reaction. This brings the reactions to 1.48 M NaCl, 13.5 µl total volume. Incubate at room temperature for 30 minutes.
5. Add 6.5 µl room temperature dilution buffer to each reaction. This brings the reactions to 1.0 M NaCl, 20 µl total volume. Incubate at room temperature for 30 minutes.
6. Add 13.5 µl room temperature dilution buffer to each reaction. This brings the reactions to 0.6 M NaCl, 33.5 µl total volume. Incubate at room temperature for 30 minutes.
7. Add 46.5 µl room temperature dilution buffer to each reaction. This brings the reactions to 0.25 M NaCl, 80 µl total volume. Incubate at room temperature for 30 minutes.
8. Store samples at 4°C.
9. Use gel shift assay to analyze samples.

Dialysis Assembly Protocol[5]

This reaction can yield a maximum of 50 pmol nucleosome in ~150 µl or 33 nM nucleosome; 36 µg/ml protein. It can be increased in scale if more material is required. The starting salt concentration is 2 M NaCl. With sequential dialysis over time, the salt concentration is lowered to 0.25 M NaCl forming the nucleosome core particle.

Materials Required but not Supplied

5 M NaCl

Dialysis units (like Pierce Slide-a-lyzer mini dialysis units 10,000 MWCO)

Dialysis buffers:

- 20 mM Tris-HCl, pH 8.0, 1.5 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-HCl, pH 8.0, 1.0 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-HCl, pH 8.0, 0.6 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-HCl, pH 8.0, 0.25 M NaCl, 1 mM EDTA, 1 mM DTT

6% Polyacrylamide gel with gel apparatus and gel buffer (ex: Invitrogen 6% DNA retardation gel)

100% Glycerol

TriDye 100 bp DNA Ladder (NEB #N3271)

1X TBE

1. Prepare 0.5 L of each of the four dialysis buffers and chill to 4°C.
2. Prepare each reaction on ice in the following order and mix well:

		For Optimizing User-supplied DNA Substrate		
	Control DNA	**0.5 to 1**	**1 to 1**	**1.5 to 1**
Water	49 µl	0 to 57 µl	0 to 54 µl	0 to 48 µl
5M NaCl	36 µl	38 µl	36 µl	32 µl
DNA	5 µl (10 µM)	50 pmol	50 pmol	50 pmol
20 µM Dimer	5 µl	2.5 µl	5 µl	10 µl
10 µM Tetramer	5 µl	2.5 µl	5 µl	10 µl
Total	**100 µl**	**100 µl**	**100 µl**	**100 µl**

The Dimer and Tetramer are supplied in 2 M NaCl.

3. Transfer the reaction to the mini dialysis units according to the manufacturers protocol.
4. Place dialysis units in 1.5 M NaCl buffer for 2–3 hours at 4°C and then transfer to each consecutively lower NaCl concentration buffer for 2–3 hours at 4°C with either the 0.6 M or 0.25 M NaCl buffer dialysis being an overnight step.
5. Transfer the samples to tubes. The volume will have increased because of the salt dialysis. Equalize sample volumes to 150 µl using the 0.25 M NaCl buffer. If volumes are off by more than 20%, some thing may have gone wrong with the set up of those samples.
6. Store samples at 4°C.
7. Use gel shift assay to analyze samples.

Gel Shift Assay

1. Mix 10 µl of each sample with 2 µl of 100% glycerol, including control reactions.
2. Load 10 µl of each sample onto a 6% DNA retardation gel. We recommend running 2 µl TriDye 100 bp DNA Ladder (NEB# N3271) alongside reactions.
3. Electrophorese according to gel manufacturer protocol in buffer containing no ethidium bromide. Using the marker lane to track the gel run, run the blue dye off the gel and the green dye about two thirds down the gel. Xylene Cyanol in these gels runs approximately where the 200 bp substrate runs.
3. Soak gel for 3 to 5 minutes in 1X TBE with ethidium bromide.
4. Analyze gel.
   - Should see a gel shift of the 208 bp band when bound by octamer to about 700 bp.
   - Free DNA stains much better by ethidium bromide than nucleosome bound complex.
   - Optimal concentration is the lowest concentration of octamer needed to see most of the DNA shifted with the least amount of higher order aggregates.
   - If not in the range, then repeat experiment increasing or decreasing the amount of octamer used until an optimal ratio is found.

DNA Concentration Formulas

To determine the concentration of binding sites:

- 1.0 A_{260} unit double stranded DNA = 50 µg/ml
- MW of double stranded DNA= # base pairs x 650 daltons/base pairs
- pmol/µl of double stranded DNA= µg/µl DNA x 10^6 pg per µg/MW
- µM double stranded DNA = pmol/µl of double stranded DNA
- Size of DNA in bp / 150 bp = # of potential binding sites
- µM of binding sites =# potential binding sites x µM DNA

Example #1: 1 kb DNA at 1 µg/µl has 6 possible binding sites = 9 µM binding site DNA solution or 9 pmol/µl binding site DNA solution.

- MW = 1000 bp x 650 daltons/base pairs = 650,000 daltons
- µM = pmol/µl = 1 µg/µl DNA x 10^6 pg per µg/650,000 = 1.5
- # of potential binding sites = 1000 bp/150 bp = 6
- conc. of binding sites = 1.5 x 6 = 9 µM or 9 pmol/µl

In the described assembly reaction for dilution assembly with 25 pmol of DNA substrate, use 2.7 µl of 9 µM binding site DNA solution per reaction and vary the amount of octamer.

Example #2: 0.5 kb DNA at 1 µg/µl has 3 possible binding sites = 4.5 µM binding site DNA solution.

- MW = 500 bp x 650 daltons/base pairs = 325,000 Daltons
- µM = pmol/µl = 0.5 µg/µl DNA x 10^6 pg per µg/325,000 = 1.5
- # of potential binding sites = 500 bp/150 bp = 3
- µM of binding sites = 1.5 µM x 3 = 4.5 µM or 4.5 pmol/µl

In the described assembly reaction for dilution assembly with 25 pmol of DNA substrate, use 5.5 µl of 4.5 µM binding site DNA solution per reaction and vary the amount of octamer.

References

1. Kornberg, R.D. (1977) *Annu. Rev. Biochem.,* 46, 931–954.
2. van Holde, K.E. (1989) *Chromatin*, 1–497.
3. Li, Y et al. (2009) *Jour. of Biol. Chem.,* 284, 34283–34295.
4. Qi, Hank H. et al. (2010) *Nature,* 466, 503–507.
5. Luger et al., (2004) *Methods in Enzymology,* 375, 23–62.

Components Sold Separately:

Histone H2A/H2B Dimer Human, Recombinant
#M2508S 2 nmol

Histone H3.1/H4 Tetramer Human, Recombinant
#M2509S 1 nmol

Nucleosome Control DNA
#N1202S 10 µM