**EpiMark® Nucleosome Assembly Kit**

**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.

**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.

**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 polyacrylamide 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 nucleosome requirement. Methods for 50 pmol and 20 µl 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
- DNA
- Water
- 10 mM Tris, pH 8.0
- 100% Glycerol
- TriDye™ 100 bp DNA Ladder (NEB #N3271)

**For 50 pmol**

- 10 µM Histone H2A/H2B Dimer
- 10 µM Histone H3.1/H4 Tetramer
- 5 µl 10 mM Tris, pH 8.0
- 50 pmol 5 M NaCl
- 20 µl DNA Substrate
- 50 pmol 100% Glycerol
- 20 µl TriDye™ 100 bp DNA Ladder
- 20 µl Water
- 40 µl Total

**For 20 µl**

- 10 µM Histone H2A/H2B Dimer
- 10 µM Histone H3.1/H4 Tetramer
- 5 µl 10 mM Tris, pH 8.0
- 50 pmol 5 M NaCl
- 20 µl DNA Substrate
- 50 pmol 100% Glycerol
- 20 µl TriDye™ 100 bp DNA Ladder
- 20 µl Water
- 40 µl Total

The Dimer and Tetramer are supplied in 2 M NaCl.

1. Incubate reactions at room temperature for 30 minutes.
2. 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.
3. 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.
4. 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.
5. 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.
6. Store samples at 4°C.
7. Use gel shift assay to analyze samples.
Dialysis Assembly Protocol

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-Cl, pH 8.0, 1.5 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-Cl, pH 8.0, 1.0 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-Cl, pH 8.0, 0.6 M NaCl, 1 mM EDTA, 1 mM DTT
- 20 mM Tris-Cl, 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)

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

Dialysis Assembly Protocol

1. Prepare the Reaction Assembly Mix on ice in the following order (for user-supplied substrate, suggested ratios have been included): 5.0 mL of Assembly Buffer, 46.5 µl 2X TBE, 13.5 µl 1X TBE, 6.5 µl 1X TBE, 3.5 µl 1X TBE, 2.5 µl 1X TBE, 1.0 µl 1X TBE, 0.5 µl 1X TBE, 0.5 µl Protein A (10 µM), 3.75 µl Octamer (10 µM), 2.5 µl Tetramer (10 µM), 2.5 µl Dimer (10 µM), 25 pmol of DNA Substrate.

2. Prepare each reaction on ice in the following order (for user-supplied substrate, suggested ratios have been included): 5.0 mL of Assembly Buffer, 46.5 µl 2X TBE, 13.5 µl 1X TBE, 6.5 µl 1X TBE, 3.5 µl 1X TBE, 2.5 µl 1X TBE, 1.0 µl 1X TBE, 0.5 µl 1X TBE, 0.5 µl Protein A (10 µM), 3.75 µl Octamer (10 µM), 2.5 µl Tetramer (10 µM), 2.5 µl Dimer (10 µM), 25 pmol of DNA Substrate.

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

1. Prepare 0.5 L of each of the four dialysis buffers and chill to 4°C.

2. Prepare each reaction in ice in the following order and mix well:

<table>
<thead>
<tr>
<th>Control DNA only (25 µmol)</th>
<th>For Optimizing User-supplied DNA Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.5 µl</td>
</tr>
<tr>
<td>5M NaCl</td>
<td>2 µl</td>
</tr>
<tr>
<td>2.5 µl (10 µM)</td>
<td>25 pmol</td>
</tr>
<tr>
<td>DNA</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>10 µl Dimer</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>20 µl Tetramer</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>Total</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

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 instructions (ex: Invitrogen 6% DNA retardation gel) 100% Glycerol TriDye 100 bp DNA Ladder (NEB #N3271) 1X TBE

4. Analyze gel.

   • Should see a gel shift of the 208 bp band when bound by octamer to about 400 bp.
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5. 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.

6. Soak gel for 3 to 5 minutes in 1X TBE with ethidium bromide.

7. Analyze gel.

   • µM of binding sites = # potential binding sites
   • µM double stranded DNA = pmol/µl of double stranded DNA
   • MW of double stranded DNA = # base pairs x 650,000 daltons/base pairs

DNA Concentration Formulas

To determine the concentration of binding sites:

- 1.0 A260 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

References


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 #N1102S 10 µM

ISO 9001
ISO 14001
ISO 13485
ISO 13485:2016
Medical Devices
and EPIMARK

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