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 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.

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 10 mM 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
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
- 50 pmol nucleosome
- 10 µg/ml DNA
- 1X TBE
- 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):
   - 20 µM Dimer
   - 5 µl
   - 2.5 µl
   - 25 pmol
   - 4 µl
   - 6 µl
   - 50 pmol

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)
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.

1. Prepare 0.5 L of each of the four dialysis buffers and 1X TBE.
2. Load 10 µl of each sample onto a 6% DNA retardation gel (ex: Invitrogen 6% DNA retardation gel).
3. Electrophorese according to gel manufacturer instructions.

DNA Concentration Formulas

To determine the concentration of binding sites:

- µM of binding sites = number of potential binding sites
- µM double stranded DNA = pmol/µl of double stranded DNA
- µM DNA = pmol/µl x 10⁶ pg per 650,000 daltons
- # of potential binding sites = 500 bp/150 bp = 3

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 x 1 µg/ml DNA x 10⁶ 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 x 0.5 µg/ml DNA x 10⁶ 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


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

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