Histone octamer, tetramer, or dimer formation

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Histones are inherently predisposed to assemble into their native complexes — H2A·H2B dimers, H3·H4 tetramers, and ultimately the histone octamer — even in complex mixtures. The formation of these complexes can be steered by the physicochemical environment. For example, at low pH, dimers and tetramers remain separate, while at neutral to slightly basic pH, electrostatic repulsion is sufficiently reduced to allow octamer formation (Sperling and Wachtel, 1981).
Under the conditions described here, monovalent ions such as chloride mimic the electrostatic shielding normally provided by DNA and maintain solubility during refolding from guanidine hydrochloride (Luger et al., 1999). This strategy tolerates crude inputs such as lyophilized or unfractionated histone mixtures, and the resulting particles can be separated by size exclusion chromatography to isolate well-defined species for downstream nucleosome reconstitution.

This is a bench card. Full protocol available online.

Procedures

›› *Nucleosome core particle reconstitution*

- ☐ Dialysis tubing, 3.5 kDa MWCO
- ☐ R0099 Unfolding buffer, 10 mL
- ☐ R0100 2 × Refolding buffer, 800 mL

(1.) Reconstitute 50 nmol of each lyophylized histone in 200 µL unfolding buffer to 250 µM (2.5–3.0 g/L).

(2.) Bring histones into solution by sonication in an ice-cooled water bath for 5 min. Then, nutate the stock solutions in the cold room for 30 min. 30 min

(3.) Clarify the solution by centrifugation at 17 000 × *g* and 4 °C for 1 min to remove any insoluble material.

(4.) Determine the protein concentration of each histone by UV/Vis spectrophotometry at 280 nm. Take multiple measurements to minimize error.

> ***Quality assurance:*** Use matched quartz cuvettes or a liquid column instrument (NanoDrop 2000). Blank with fresh unfolding buffer. For liquid column measurements, increase drop size to 2 µL as surface tension is low due to high guanidine content.

(5.) Prepare sample to a final concentration of 10 µM particle concentration (octamer, tetramer, or dimer):

Histone	Stock	Ratio	Octamer	Tetramer	Dimer
H2A	250 µM	1.08	190 µL	Not applicable	176 µL
H2B	250 µM	1.08	190 µL	Not applicable	176 µL
H3	250 µM	1.00	176 µL	176 µL	Not applicable
H4	250 µM	1.00	176 µL	176 µL	Not applicable
Unfolding buffer	1 ×		1 468 µL	1 848 µL	1 848 µL

(6.) Transfer the histone mixture to a 3.5 kDa MWCO dialysis cassette using a 18 G or 21 G needle. Dialyze against at least 100 mL 1 × refolding buffer for 2 h at 4 °C. Stir the dialysis buffer gently. 6 h

> ***Critical:*** This step removes guanidine and allows monovalent salt to drive refolding of the histone complexes. Use at least a 50 vol excess of buffer relative to sample volume to ensure efficient buffer exchange.

(7.) Repeat the dialysis step two more times.

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›› *Sample preparation for size exclusion chromatography (SEC)*

(1.) Clarify the dialyzed sample by centrifugation at 17 000 × *g* for 5 min at 4 °C.

> ***Critical:*** Do not skip this step. Aggregates may clog the ultrafiltration device or interfere with SEC.

(2.) Equilibrate a disposable ultrafiltration device with 1 × refolding buffer at 12 000 × *g* for 5 min at 4 °C.

(3.) Transfer the cleared sample into the ultrafiltration device. Concentrate at 12 000 × *g* for 5 min intervals to reach 250–500 µL. Between spins, gently pipette or flick the concentrate to prevent edge-aggregation.

> ***Critical:*** Do not exceed the manufacturer's recommended centrifugal force, typically 14 000 × *g*, as this can rupture the membrane and result in sample loss.

(4.) Equilibrate a 0.45 µm spin filter with refolding buffer and filter the concentrated sample to remove aggregates or particulate matter.

> ***Quality assurance:*** The overall recovery after concentration and filtration is typically 70–90%, depending on histone quality, aggregation, and labeling. Highly concentrated or dye-labeled samples may require gentler handling or multiple clarifications to avoid loss. Avoid concentrating samples beyond 100–150 µM unless needed for low-volume injections.

›› *Size exclusion chromatography of histone complexes*

(1.) Prepare histone samples for injection by clearing, concentrating, and filtering as described above.

(2.) Inject the sample onto a preparative Superdex™ 200 column equilibrated with 2.5 column volumes refolding buffer.

> ***Critical:*** Never flow refolding buffer without equilibrating the FPLC system in water! The system is stored in 20% ethanol and salt will crash out and ruin the column.

(3.) Elute the particles by flowing 1.4 column volumes refolding buffer.

> ***Quality assurance:*** For octamers, expect a sharp, symmetric peak eluting at 13.5–14.0 mL depending on exact conditions and sample quality. Aggregates or subcomplexes appear earlier or later, respectively.

(4.) Collect and analyze fractions around the main peak by SDS-PAGE to confirm stoichiometry and purity.

> ***Quality assurance:*** Octamer-containing fractions typically show near-equal band intensities for all four histones.

Storage of histone dimers, tetramers, and octamers

(1.) Combine desired fractions, quantify by UV absorbance at 280 nm using the extinction coefficients below. Concentrate over an ultrafiltration device to 72 µM for octamers or 144 µM for dimers as before.

(2.) Add an equal volume of 100% glycerol to make up final stocks to 72 µM (36 µM for dimers or tetramers). Nutate at 4 °C for 30 min.

> ***Critical:*** Before reconstituting nucleosomes from these 50% glycerol stocks, dialyze against refolding buffer overnight at 4 °C.

(3.) Aliquot and store at −20 °C.

List of references

K. Luger, T. Rechsteiner, and T. Richmond, *Methods Mol. Biol.* **119** 1—16 (1999).

R. Sperling and E. Wachtel, *Adv. Protein Chem.* **34** 1—60 (1981).