Histone octamer, tetramer, or dimer formation

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.

Risk assessment

▷ Wear gloves, safety glasses, lab coat

Reviewed: Mar 27, 2025

Procedures

›› *Nucleosome core particle reconstitution*

- ☐ Dialysis tubing, 3.5 kDa MWCO
- ☐ Unfolding buffer, 10 mL ⟨*R*⟩
- ☐ 2 × Refolding buffer, 800 mL ⟨*R*⟩

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

> ***Note:*** Extinction coefficients to determine histone concentrations are provided as Supplementary Resources.

(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

> ***This is why:*** Although the histones are denatured by guanidine, cooling during sonication minimizes heat-induced aggregation and preserves reducing conditions.

(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.

> ***This is why:*** Although histones lack tryptophan, tyrosine and phenylalanine residues contribute enough absorbance for reliable quantification under denaturing conditions. Guanidine has minimal absorbance at 280 nm.

(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

> ***Hint:*** The table gives examples for 22 nmol octamer and 48 nmol dimer (or tetramer) formation in 2.2 mL denaturing buffer, assuming 250 µM stock solutions. Most likely, one of the histones will limit the total amount that can be assembled. Scale accordingly.

> ***This is why:*** A small excess of H2A and H2B improves octamer formation and minimizes tetramer contamination in the octamer peak when the sample is polished by size exclusion chromatography.

(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.

›› ***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.

> ***Hint:*** Use a low protein-binding membrane such as cellulose acetate (CA). For particles containing fluorophore-labeled histones, check compatibility with the filter material.

> ***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.

> ***Note:*** Typical injection volumes range from 100–500 µL. Do not exceed the loop size of the column.

(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.

> ***Hint:*** On a preparative Superdex™ 200 column and at a flow rate of 0.2 mL/min, collect 250 µL fractions. Dilute 3 µL of each fraction in 6 µL water to reduce salts, then add 3 µL 4 × SDS loading dye. Analyze 10 µL per lane on a 16% acrylamide gel.

> ***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.

Troubleshooting

Sample preparation for size exclusion chromatography (SEC)

In Step 4:

– Sample loss when using cyanine-labeled (Cy3, Cy5, etc.) histones.

  ◦ Instead of spin filtration, clarify the sample by centrifugation at 17 000 × *g* for 10 min and transfer the supernatant to a fresh tube. Repeat once if needed.

Recipes

Unfolding buffer, pH 7.4

Amount	Ingredient		Stock	Final
200 µL	Tris-Cl, pH 7.4	R0055	1 M	20 mM
5.73 g	Guanidine, · HCl, ultrapure [50-01-1]		95.53 g/mol	6 M
100 µL	Dithiothreitol (DTT) □	R0015	1 M	10 mM
10 µL	Ethylenediaminetetraacetate (EDTA), pH 8.0	R0017	0.5 M	0.5 mM
To 10 mL	Water, reagent-grade			

Prepare fresh. ***Quality assurance:*** Use ultrapure guanidine hydrochloride.

Unfolding buffer

20 mM Tris-Cl, 6 M Guanidine, □ 10 mM DTT, 0.5 mM EDTA, pH 7.4

WARNING

Eye irritation; Skin irritation

Date: Sign: R0099

Refolding buffer, pH 7.4, 2 ×

Amount	Ingredient		Stock	Final
20 mL	Tris-Cl, pH 7.4	R0055	1 M	20 mM
234 g	NaCl	[7647-14-5]	58.44 g/mol	4 M
2 mL	Dithiothreitol (DTT) □	R0015	1 M	2 mM
2 mL	Ethylenediaminetetraacetate (EDTA), pH 8.0	R0017	0.5 M	1 mM
To 1 L	Water, reagent-grade			

Filter sterilize. Add reducing agent right before use. Store at 4 °C.

2 × Refolding buffer

10 mM Tris-Cl, 2 M NaCl, □ 1 mM DTT, 0.5 mM EDTA, pH 7.4 *[At 1 × dilution]*

WARNING

Eye irritation; Skin irritation

Date: Sign: R0100

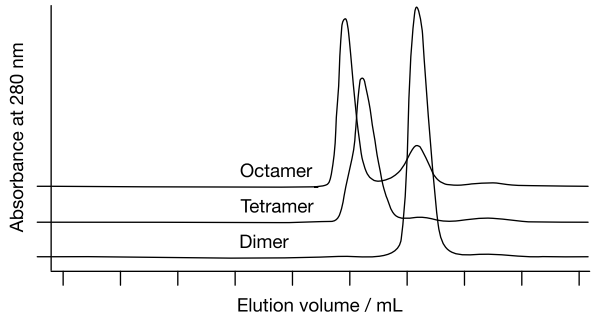
Resources

Nucleosome core particle reconstitution

Histone	Mol. Abs. Calculated	Empirical	Difference	Species	Reference
H2A	4 470 $M^{-1}\,cm^{-1}$	4 050 $M^{-1}\,cm^{-1}$	+10.4%	*B. taurus*	D'Anna and Isenberg (1974)
H2B	7 450 $M^{-1}\,cm^{-1}$	6 700 $M^{-1}\,cm^{-1}$	+11.2%	*B. taurus*	D'Anna and Isenberg (1974)
H3	4 470 $M^{-1}\,cm^{-1}$	4 040 $M^{-1}\,cm^{-1}$	+10.6%	*B. taurus*	D'Anna and Isenberg (1974)
H4	5 960 $M^{-1}\,cm^{-1}$	5 400 $M^{-1}\,cm^{-1}$	+10.4%	*B. taurus*	D'Anna and Isenberg (1974)
Dimer, (H2A·H2B)	11 920 $M^{-1}\,cm^{-1}$	10 750 $M^{-1}\,cm^{-1}$	+10.9%	–	Summed from D'Anna and Isenberg (1974)
Tetramer, $(H3{\cdot}H4)_2$	20 860 $M^{-1}\,cm^{-1}$	18 880 $M^{-1}\,cm^{-1}$	+10.5%	–	Summed from D'Anna and Isenberg (1974)
Octamer	44 700 $M^{-1}\,cm^{-1}$	39 630 $M^{-1}\,cm^{-1}$	+12.8%	–	Summed from D'Anna and Isenberg (1974)
Octamer	44 700 $M^{-1}\,cm^{-1}$	45 000 $M^{-1}\,cm^{-1}$	+6.7%	*H. sapiens*	Prevelige and Fasman (1987)

In Step 1: Comparison of calculated and empirical molar extinction coefficients at 280 nm for reduced denatured histones or their assembled complexes. *Bos taurus* calf thymus extracts are consistent with the aromatic content of histones from *Xenopus laevis* and *Homo sapiens*. Calculated values use standard absorbance coefficients for tyrosine and phenylalanine. No known histone contains tryptophan. The calculated molar absorptivity is the common practical value in the chromatin field.

Size exclusion chromatography of histone complexes



In Step 3: Elution profile of histone subcomplexes from a Superdex™ 200 column. The histone octamer (108.5 kDa) elutes first, separate from a small H2A·H2B dimer excess (27.0 kDa). In contrast, the $(H3{\cdot}H4)_2$ tetramer (53.0 kDa) elutes close to the octamer peak. Note the small shoulder indicative of some octamer-like assemblies formed by the tetramer. Modified from Dyer et al. (2004).

List of references

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Change log

2020-06-12	Bradley J. Lukasak	Initial version.
2020-07-12	Hai T. Dao	Updated sample preparation for FPLC.
2022-09-11	Benjamin C. Buchmuller	Adaptation as SOP; convert to molar concentrations.
2025-03-27	Benjamin C. Buchmuller	Revised size-exclusion protocol.

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