PROTOCOL: GCP Protein Quantification Assay and QC (QUANT)

Purpose

To determine overall protein yield and histone purity after histone extraction.

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SDS-PAGE gels are not required, only when changing variables to histone extraction
protocol or something occurred questioning histone purity
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Preparation

1. Label as many 0.6 mL microcentrifuge tubes {QUANT-M13} as needed for samples being checked by SDS-Page gel
2. If you have less than 48 samples, label as many 0.6 mL microcentrifuge tubes {QUANT-M13} as needed for Protein Quantification Assay
3. Thaw resuspended histone samples on ice

Materials

- HPLC-grade water {QUANT-M01}
- NuPAGE 4x Sample Load Dye {QUANT-M02} [Life Technologies, NP0008]
- NuPAGE 4-12% gradient Bis-Tris precast gel (10 well/15 well) {QUANT-M03} [Life Technologies, NP0335BOX/NP0336BOX]
- SeeBlue Plus 2 Prestained Standard {QUANT-M04} [Life Technologies, LC5925]
- NuPAGE MES Running Buffer {QUANT-M05} [Life Technologies, NP0002]
- Simply Blue Safe Stain {QUANT-M06} [Life Technologies, LC6065]
- 2mg/mL Albumin Standard {QUANT-M07} [Thermo Scientific, 23209]
- Coomassie Plus Protein Assay Reagent {QUANT-M08} [Thermo Scientific, 23236]
- 96-well PCR plate {QUANT-M09} [VWR, 82006-704]
- Clear, flat bottom assay plate {QUANT-M10} [Greiner Bio-One, 655101]
- 50 mL Reagent Trough {QUANT-M11} [Costar, 48470]
- 1.7 mL Microcentrifuge Tubes {QUANT-M12} [Axygen, MCT-175-L-C]
- 0.6 mL Microcentrifuge Tubes {Quant-M13} [Axygen, MCT-060-L-C]
- Parafilm {QUANT-M14}
- 500 mL glass graduate cylinder {QUANT-M15}
- De-ionized water {QUANT-M16}
- Axygen Foil Seal {QUANT-M17} [Axygen, PCR-AS-200]

Assets

- SDS-PAGE Gel Box Apparatus {QUANT-A01}
- SDS-PAGE Gel Box Dam
- Voltage Box {QUANT-A02}
- Spatula {QUANT-A03}
- Gel Staining/De-staining Box {QUANT-A04}
- Gel Rocker {QUANT-A05}
- Gel Imager {QUANT-A06}
- UV-Vis Spec {QUANT-A07}
- Heat block {QUANT-A08}

- Bench Vortex {QUANT-A09}
- Mini-centrifuge {QUANT-A10}
- 12-channel pipette {QUANT-A11}
- single-channel pipettes {QUANT-A12}

Reagent Mixes

ID	Name	Step	Composition	Volume	Use
QUANT-MIX 01	SDS-PAGE loading mastermix	QUANT	25% {QUANT-M02} 62.5% {QUANT-M01}	As much as needed- see worksheet	Sample loading buffer for an SDS-PAGE gel. Weighs protein down in the gel, and allows visibility for loading
QUANT-MIX 02	1x Sample Load Dye	QUANT	25% {QUANT-M02} 75% {QUANT-M01}	1 mL	Load into empty wells of SDS-PAGE gel for even bands
QUANT-MIX 03	1:20 MES running buffer	QUANT	5% {QUANT-M05} 95% {QUANT-M02}	500 mL	Running buffer used in SDS-PAGE gel

NB: Reagent mixes are made at the time they are needed

Procedure

SDS-PAGE Gel:

1. Prepare as much QUANT-MIX01 as needed. For a full plate (96 samples) prepare at least 770 uL.
    a. MIX01 Prep:
        ❒ 2uL {M02} x _____ samples=_____ uL
        ❒ 5 uL {M01}x_____samples=_____ uL
        Add to 1.5 mL tube. Vortex to mix

NB: Calculate volumes for at least 15 more samples than you have so you won't get caught short

2. Prepare QUANT-MIX02
    a. QUANT-MIX02 Prep:
        ❒ 750 uL {M01}
        ❒ 250 uL {M02}
        Add to 1.7 mL microcentrifuge tube {QUANT-M12}. Vortex to mix. Store at bench.
3. Add 7 uL of QUANT-MIX01 to each labeled 0.6 mL tube.
4. Vortex and spin down thawed samples. Add 1 uL sample to labeled tube containing {QUANT-MIX01}
5. Spin down tubes so sample is at the bottom of tube. Incubate samples at 96°C for 10 min on heat block {QUANT-A07}.
6. While samples incubate, assemble SDS-PAGE Gel Box apparatus {QUANT-A01} with precast gels {QUANT-M03}
    a. Prepare QUANT-MIX03:

i. Acquire 500 mL glass graduated cylinder {QUANT-M15}
ii. Add 25 mL MES Running Buffer {QUANT-M05} to cylinder
iii. Fill up to 500 mL with de-ionized water {QUANT-M16}
iv. Cover top in Parafilm {QUANT-M12} and invert 7 times to mix

b. Pour QUANT-MIX 03 into the inner chamber to the top, check to make sure no leaks form
c. Pour remaining MIX03 into the outer reservoir
d. Remove combs from precast gels

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NB:Take care removing the combs.  Pull and wiggle comb gently to remove.  If too much force is used you can rip a hole in the gel
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7. Remove samples from heat block {QUANT-A01}. Let cool and spin down
8. Acquire {QUANT-M04}
9. With gel-loadng tips, load 8 uL of {QUANT-M04} into the first well. Load the rest of your samples in subsequent wells. Load empty wells with 8 uL {QUANT-MIX02}

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Gel Map: Record Sample Locations Here

Front Gel: ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___

Back Gel:  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___  ___
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10. Place top on gel box, making sure contacts are made
11. Turn voltage box {QUANT-A02} on, run gel at 150V for 45 min.
    a. After 45 min, check status of gel front, may have to run gel 5 min more for gel front to reach the bottom
12. While gels run prepare gel staining/de-staining boxes {QUANT-A04} by acquiring top and bottom. Label lid with initials, date, study, and front/back gel.
13. Once gel front reaches the bottom, turn voltage box off. Remove lid and pour running buffer down the drain
14. Remove the front gel from the inner chamber. Rinse in de-ionized (d.i) water {QUANT-M16}
15. Use a spatula {QUANT-A03} to crack open the plastic. Orient gel so the ladder is on the right side. Remove the top piece of plastic
16. Transfer gel to labeled staining box by flipping cassette over and peeling gel off cassette with the aid of the spatula. Once gel is in the box rinse once more with d.i water
17. Add 3 pumps of {QUANT-M06} to the box. Place lid on top and place setup on the rocker {QUANT-A05}. Rock on the lowest setting for 4 hours till overnight
18. Pour stain {QUANT-M06} down the drain. Rinse gel with d.i water {QUANT-M16}, then fill box slightly with d.i water {QUANT-M16}. Place setup back on the rocker {QUANT-A05} to destain overnight
19. Once gel is destained pour d.i water down drain, add a small amount of fresh water so you can easily manuever the gel in the box
20. Image gel on GelDoc
    a. Save file to folder “Histone QC” in Study folder
    b. include date, name of study, and which gel this is in name of image

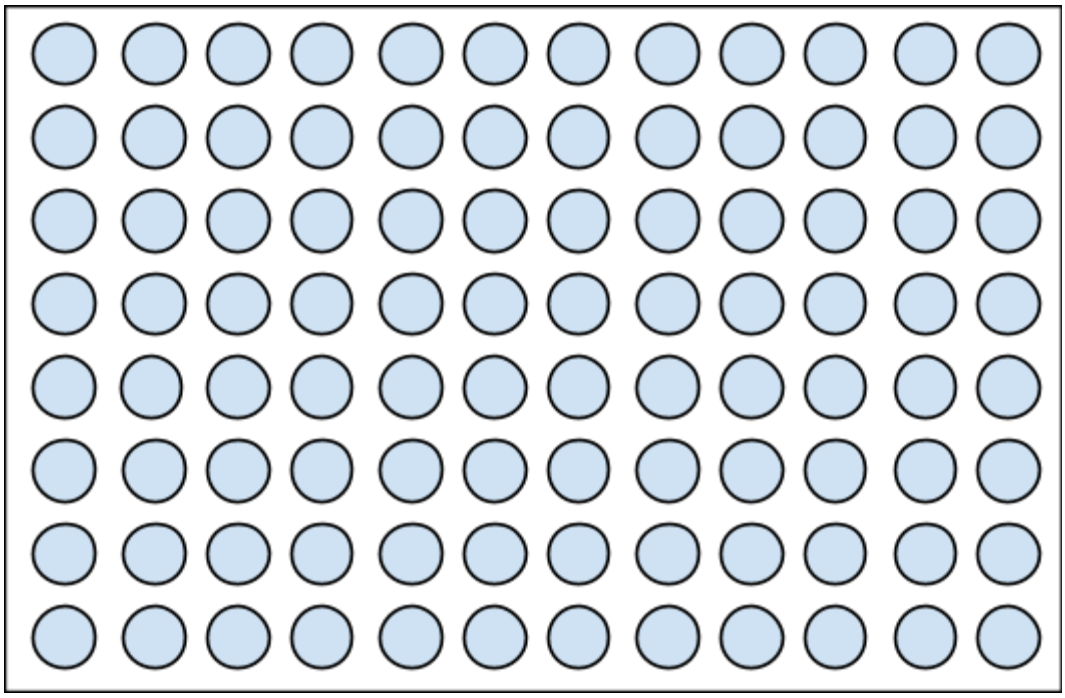
Protein Quantification Assay

1. With {QUANT-M07}, prepare a 7-point standard curve, with {QUANT-M01} as the diluent:
    a. Preparation of Standard Curve:

Vial	Vol. (uL) of Diluent {QUANT-M01}	Vol. (uL) and Source of Albumin	Concentration (ug/mL)
A	200	200 Albumin Stock {QUANT-M07}	1,000
B	200	200 Vial A	500
C	200	200 Vial B	250
D	200	200 Vial C	125
E	200	200 Vial D	62.5
F	200	200 Vial E	31.25
G	200	None	0 (Blank)

NB: Vortex and spin each vial down before creating next vial, so concentration as accurate as possible

2. If conducting this assay for a full plate of samples (96 samples) acquire 1 VWR PCR plate {QUANT-M09} , if testing less than 48 samples get previously labeled 0.6 ml tubes
3. Dilute samples 1:10 in plate/tubes by adding 18 uL HPLC-grade water {QUANT-M01} to well and 2 uL sample.
    a. Dilution Plate Map



NB: During histone extraction/resuspension steps you may notice some samples may be more concentrated/dilute than other samples/previous sample sets. If you feel that a 1:10 dilution will result in reads outside the standard curve, dilute more/less than 1:10. If samples need to be more concentrated, create enough so that there can be

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duplicate reads (>10 uL)

Sample Dilution: _______ Water: _______uL    Sample: _____ uL
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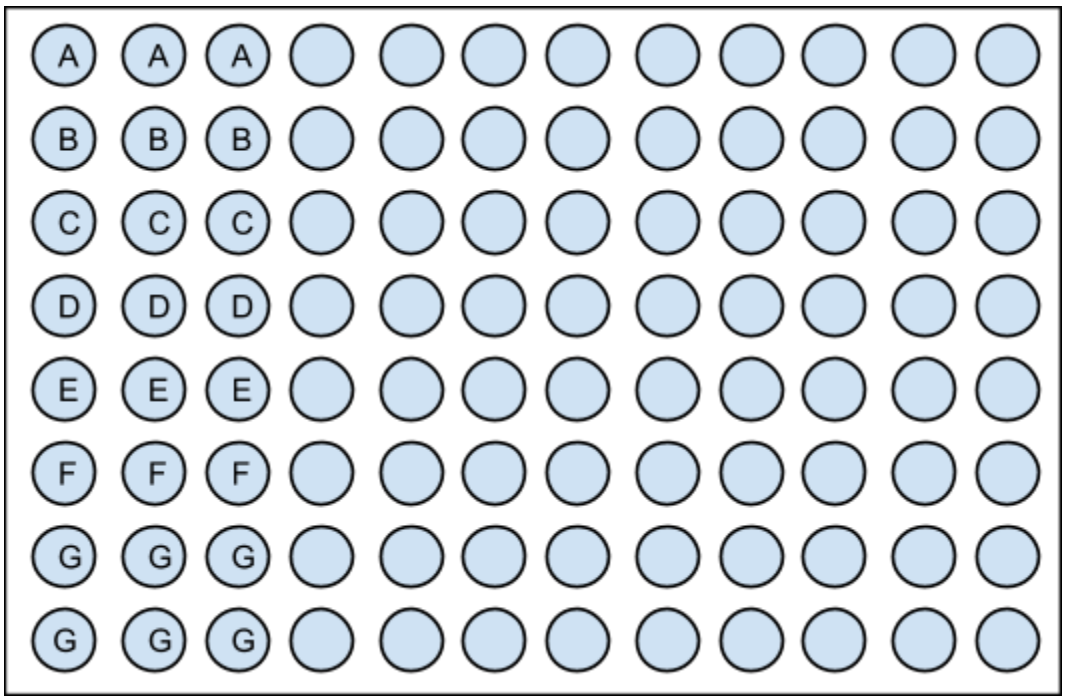
4. Plate standard curve in clear flat-bottom 96 well assay plate {QUANT-M10}. Plate Vials A through G in the first three columns, 5 uL/well
    a. Plate Map:



5. Plate samples in triplicate, 5 ul/well. For a full plate this will need 3 clear, flat-bottom 96-well plates.
    a. Plate Maps:

Plate1

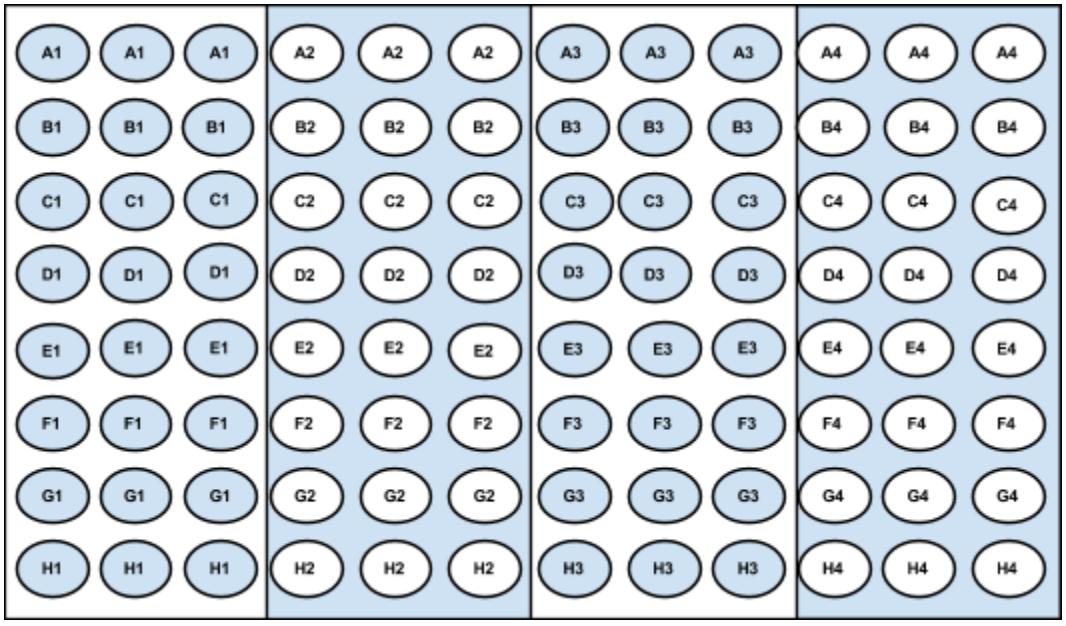


Plate 2

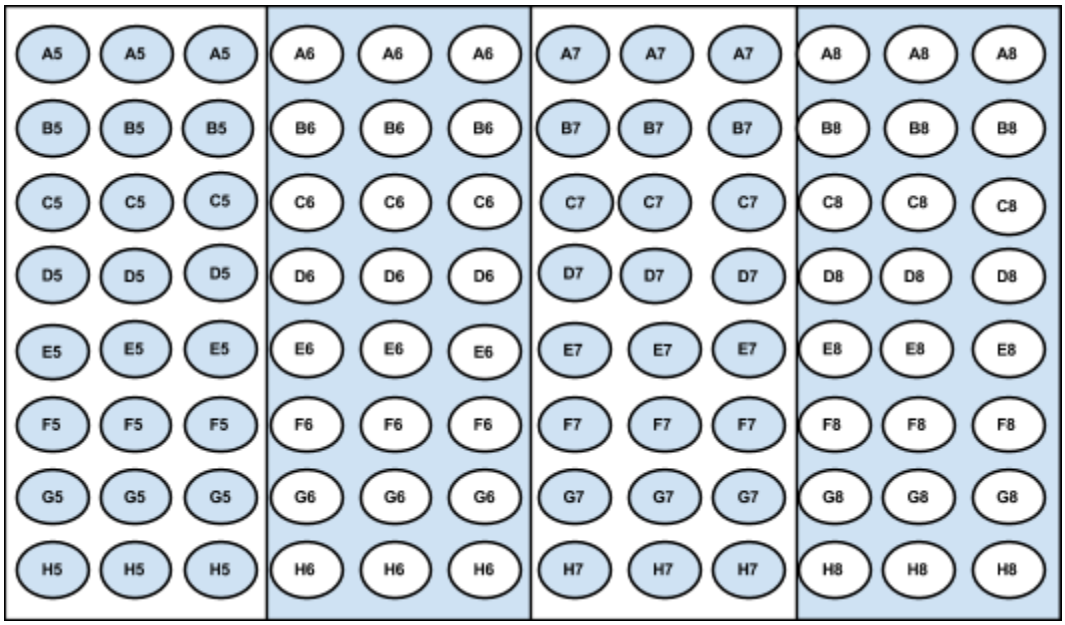
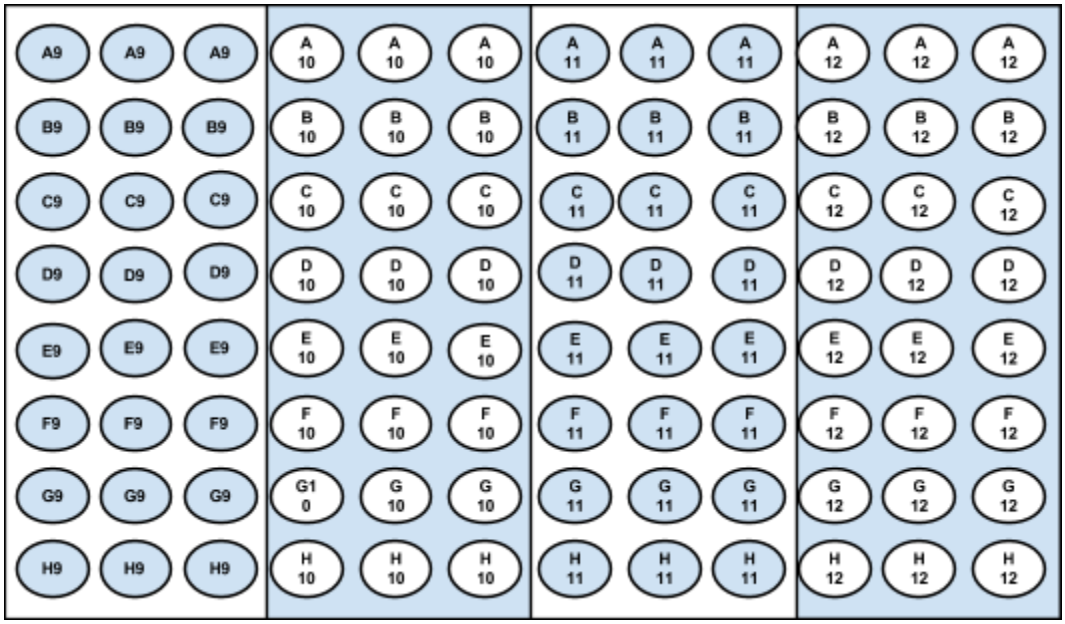


Plate 3



6. Add 195 uL Coomassie Plus Protein Reagent {QUANT-M08} to each well

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NB: Use 12 channel multichannel pipette.  Dispense reagent to the first stop in the
pipette so as to not introduce bubbles.
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7. Place foil seal {QUANT-M17} on top of each plate and take plates to UV/Vis spectrophotometer {QUANT-A07}
8. Open software SoftMax Pro v. 6.3. Load “LINCS GCP Protein Quantification Assay_Manual” found in LINCS GCP folder.
9. Save file with date and name of current study into your specific study folder
10. On the right-hand side of the screen you will see plates groups labeled “Standard Curve”, “Plate1” “Plate2”, and “Plate3”. Remove foil from Standard Curve plate and place in drawer, close the drawer

11. Highlight “Standard Curve Plate” on Software and click “Read”. Spectrophotometer will shake the plate for 10sec and then take the readings. Repeat for plates 1-3.

NB: Upon clicking “Read” software will warn that you are about to replace data for that plate. Click “Ok”

12. Once all plates are read scroll down to box with standard curve readings and curve fit information. Change the curve fit for the Standard Curve to “Quadratic”
13. Save file again.
14. Click “File”→”Export Data” to same folder file is saved in
    a. Check “all plates”, “all groups”, “raw”, and “reduced” data
    b. For output format click “plate”, OK, and save as a .xls file
    c. This information will be used to determine volumes of sample used in “Primary Propionylation”