PROTOCOL: GCP Histone Extraction (HE)

Purpose

To extract histones from frozen cell pellets. The lysing steps in this protocol lyse the cell while keeping the nuclei intact. Additives added to the base of the lysis buffer are HDAC inhibitors, phosphatase inhibitors, and protease inhibitors to help keep the histone PTMs in tact during lysis. DTT is added to reduce disulfide bonds. Placing the resulting pellet in a dilute sulfuric acid solution extracts histones, as they are soluble due to multiple charges from lysine residues, while other cellular material stays out of solution. A 60% w/v trichloroacetic acid solution precipitates the histones from this solution, allowing them to be isolated.

Preparation

1. Thaw samples on ice.
2. Prepare HE-BUF01-keep on ice
3. Prepare HE-MIX01
4. Label 0.6 mL microcentrifuge tubes {HE-M05} so that each sample has a tube
5. Set centrifuge {HE-A02} to 4°C by setting temperature and pressing “Fast Cool”

```
Note: If any reagents used to prepare HE-BUF01 are low/depleted, refer to protocol
“GCP Lysis Buffer and Additives Prep (LBAP)” to make more
```

Materials

- Nucleus Buffer {LBAP-BUF01}
- 500 mM pre-aliquoted Dithiothreitol (DTT) {HE-M01} [Thermo, 20291]
- 2.27M Sodium Butyrate {LBAP-ADD06} [Sigma, B5887]
- 250 mM AEBSF {LBAP-ADD07} [Calbiochem, 101500]
- 5 uM Microcystin LR {LBAP-ADD08} [Calbiochem, 475815]
- 10% NP40 {LBAP-ADD09} [USB Corporation, 19628]
- HPLC-grade Water {HE-M02}
- H_2SO_4 {HE-M03} [Sigma, 339741-100mL]
- Trichloroacetic acid {HE-M04} [BDH, BDH0310-500G]
- 0.6 mL microcentrifuge tubes {HE-M05} [Axygen, MCT-060-L-C]
- Capless 1.5mL Sarstedt microcentrifuge tubes {HE-M06} [Sarstedt, 72.692]

Assets

- Bench-top vacuum trap {HE-A01}
- Bench-top Eppendorf Centrifuge 5417R {HE-A02}
- Bench-top vortex with removable shaking platform {HE-A03}

Reagent Mixes

ID	Name	Step	Composition	Stock Volume	Use
HE-BUF01	Lysis Buffer	HE	1mM DTT {HE-M01}, 10 mM {LBAP-ADD06},	As much as needed	To isolate nuclei from cells

			0.5 mM {LBAP-ADD07}, 5 nM {LBAP-ADD08}, 0.3% {LBAP-ADD09} in {LBAP-BUF01}		
HE-MIX01	0.4N H_2SO_4	HE	109 uL {HE-M03} in 10mL {HE-M02}	As much as needed	To extract histones from nuclei
HE-MIX02	60% w/v Trichloroacetic acid (TCA)	HE	60% TCA in {HE-M02}	as much as needed	To crash histones from acid solution

Reagent Mix Preparation

NB: HE-MIX02 (60% w/v TCA) is made just before use on the second day of the procedure

HE-BUF01: Lysis Buffer

1) Use the following table to prepare as much buffer as needed for the experiment. Make at least 100 mL for a full plate (96 samples).

Reagent	Location	Volume for 10 mL Buffer	Volume for ____mL Buffer
Nucleus Buffer {LBAP-BUF01}	4°C Walk-in	10 mL	
500 mM DTT {HE-M01}	Dessicator by weigh station	20 uL	
2.27M Sodium Butyrate {LBAP-ADD06}	-20°C additives box	4.4 uL	
250 mM AEBSF {LBAP-ADD07}	-20°C additives box	20 uL	
5 uM Microcystin LR {LBAP-ADD08}	-20°C additives box	10 uL	
10% NP40 {LBAP-ADD09}	-20°C additives box	300 uL	

- ❏ To make 500 mM DTT {HE-M01}, add 100 uL HPLC-grade water {HE-M02} to tube with pre-aliquoted material. Pipette up and down until fully resuspended. Repeat until have enough solution for the lysis buffer
- ❏ Chill container being used for the lysis buffer {HE-BUF01} on ice.
- ❏ Add nucleus buffer {LBAP-BUF01} to chilled container. Follow with the rest of the additives.

Note: All additives can be thawed at room temperature. Microcystin LR {LBAP-ADD08} can take a longer time to thaw.

HE-MIX01: 0.4N H_2SO_4

1) Determine how much 0.4N H_2SO_4 {HE-MIX01} is needed for the experiment (over calculate by 10 samples):
   - ❒ _____samples x 400 uL 0.4N H_2SO_4 {HE-MIX01}= _____ uL 0.4N H_2SO_4 {HE-MIX01}
   - ❒ Add 109 uL H_2SO_4 {HE-M03} to every 10 mL HPLC-grade water {HE-M02}
   - ❒ Vol. HPLC-grade water {HE-M02} used:_____ Vol. H_2SO_4 {HE-M03} used: _____

Procedure

Nuclei Isolation and Histone Acid Extraction

1. Resuspend pellet in 500 uL Lysis Buffer {HE-BUF01} by via gentle vortexing. If the pellet can not be resuspended, switch to manual suspension via pipette. Make a note of changes:

2. Spin samples for 1 min at 10,000g, 4℃
3. Remove supernatant with vacuum trap {HE-A01}. Make sure to aspirate by having pipette tip touch just the surface of the supernatant.
4. Repeat cell lysis (steps 1-3) once more

NB: Some cell types may require fewer or more iterations of the cell lysis procedure. Record deviations from protocol, if any occur here:

5. Resuspend pellet in 400uL 0.4N H_2SO_4 {HE-MIX01} via gentle vortexing. If the pellet does not go into solution, switch to manual resuspension by pipetting up and down. Make a note of any changes that occur at this time:

6. Place samples on bench-top shaker {HE-A03} at room temperature. Shake gently overnight.

Histone Precipitation and Isolation

1) Set centrifuge to 4℃
2) Prepare 60% w/v trichloroacetic acid (TCA) {HE-MIX02}:

   - ❒ _____samples x 200 uL 60% w/v TCA {HE-MIX02}= _____ uL {HE-MIX02}
   - ❒ For every 5 mL of 60% w/v TCA solution weigh out 3g of TCA {HE-M04} . Mass of TCA:_____
   - ❒ Bring up TCA to the total volume in HPLC-grade water {HE-M02}. Add {HE-M02} in small amounts at a time, vortexing in between to dissolve the TCA {HE-M04}**.** Add enough water to bring solution up to total volume.

3) Remove samples from platform shaker. Pellet samples for 5min, 10,000 g, 4℃
4) Transfer supernatant to a new, labeled tube from the previous day, on ice
5) Add 200 uL 60% w/v TCA {HE-MIX02} to supernatant. Vortex briefly.
6) Incubate samples on ice for 30 min. Time Start:_____ Time Finished:_____
7) Centrifuge sample at 15,000 rcf for 20 min, 4℃

8) Remove supernatant with vacuum trap, taking care not to disturb the film. If you see the film starting to detach from the wall of the tube re-spin for 10 min at 15,000 rcf, 4℃
9) Gently pipette 100 uL chilled 100% acetone on the side of the tube opposite of film. Carefully roll acetone around tube once. Try not to disturb the film
10) Spin samples for 10 min at 15,000, 4℃.
11) Remove supernatant with vacuum trap.
12) Allow samples to air dry with the caps open at room temperature for 15 minutes. This can be done by place samples under a hood with the air flow on. Make sure all of the acetone has evaporated. Cap and place samples at 4℃ if resuspending samples that day, or place at -80℃ if resuming at a later date
13) Resuspend dried histone films in 15 uL HPLC-grade water {HE-M02}, on ice. Resuspend the film manually, by pipette, by moving the droplet of water down the film several time, untill no more film is visible. Vortex and spin down.

NB: Some pellets may yield so much histone, that 15 uL of water is not enough, and a particulate will be visible at the bottom of the tube after centrifugation. Add more water in increments of 5 uL to help dissolve extra film. If film does not dissolve residue may not be histone, but extra material co-purified with the histones. Do not add any more water to help dissolve this residue. If extra water is added to sample, record here: