Histone PTM Quantitation Service Cell Preparation Protocol

NOTES: Accurate cell counts and assessment of cell viability are essential for the generation of high quality data and must be provided on the sample submission form. A minimum of 500,000 cells per sample is required and should be provided without residual traces of serum, media or PBS which if present, will compromise data quality.

Please include two extra samples that are representative of your test samples. These samples will be used by Active Motif to determine the optimal histone extraction method for your project.

Adherent cells

1. Aspirate media and wash cells with PBS.
2. Detach cells from the culture dish according to the same method used for passage using a sufficient amount of trypsin or equivalent.
3. Suspend cells in 1 ml of their growth medium and transfer to a 1.5 ml microfuge tube.
4. Continue with the procedure for non-adherent cells below.

Non-adherent cells

1. Perform cell counts and determine cell viability.
    - For cell viability, Trypan blue (mixed 1:1 with 10 ul of cells) is suggested.
    - Record cell counts and viability on the Sample Submission form.
2. Pellet cells at 150 x g for 5 min.
3. Aspirate media and gently resuspend pellet in 1 ml PBS.
4. Pellet the cells at 150 x g for 5 min.
5. Repeat the PBS wash (steps 3 and 4) twice for a total of three washes.
6. Aspirate PBS, leaving behind ~50 - 100 ul PBS.
7. Using a P200 pipette, remove all traces of PBS.
8. Immediately cap tube and snap freeze on dry ice or in liquid nitrogen.
9. Store cell pellets at -80°C.
10. Ship cells on dry ice. Fill out Active Motif's **Sample Submission Form**, include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

Adherent cells in 6,12 or 24 well plates

Please include two extra samples that are representative of your test samples on a separate plate for Active Motif's determination of the optimal histone extraction method for your project.

1. Seed cells into the multi-well plates. Prepare an extra plate or additional wells for cell counts and viability determination.
2. Perform cell counts and viability determination using the extra plate or wells prepared for this purpose.
3. For plates to be shipped to Active Motif, remove lid and number plate(s) and wells of each plate that contains cells.
4. Aspirate media and wash cells with maximum allowable volume of cold PBS.

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5. Aspirate PBS.
6. Perform two additional PBS washes for a total of three washes.
7. With the plate resting on its lid at an angle, allow residual PBS to collect at well bottom for 1 min.
8. Aspirate to remove all traces of PBS.
9. Attach adhesive film and replace cover. Attach cover to plate with sufficient tape to ensure lid will remain attached during shipment.
10. Immediately place plate at -80 °C or on dry ice.
11. Store plate at -80°C.
12. Ship cells on dry ice. Fill out Active Motif's **Sample Submission Form**, include a hard copy with your samples and ship according to the instructions on the Sample Submission Form.

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