PROTOCOL: Primary Propionylation (K1)

Purpose

To propionylate lysine residues at the protein level for later mass spectrometric analysis.

Preparation

1. Calculate the volume of histone needed to reach 10ug per sample.

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NB: See Spreadsheet “Primary Propionylation Calculations” found here: “Histone-MRM\Primary Propionylations Calculations and Sample Plate Map”. Populate with samples, fill out sample layout, and perform calculations to determine volumes of reagents and samples needed.
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2. If using SILAC standardization, calculate the volume of histone needed to reach 5ug per sample and 5ug of heavy standard triple mix.

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NB: Heavy standard triple mix contains an equal amount in ug of three cancer cell lines (HeLa, K562, and 293T).
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3. Prepare MIX01 (500mM Sodium Phosphate, Monobasic)
4. Prepare MIX02 (500mM Sodium Phosphate, Dibasic)
5. Prepare MIX03 (500mM Sodium Phosphate Buffer, pH 8)
6. Prepare MIX04 (0.1% TFA)
7. Prepare MIX05 (400nmol/uL NHS Propionate in Methanol)

Materials

- HPLC-grade water {K1-M01} [JT Baker, 4218-03]
- Anhydrous Methanol {K1-M02} [JT Baker, 9097-12]
- Trifluoroacetic Acid {K1-M03} [Sigma-Aldrich, T6508-25ML]
- NHS-Propionate {K1-M04} [SAI Chemicals]
- Sodium Phosphate, Monobasic {K1-M05} [Sigma-Aldrich, P0662-500G]
- Sodium Phosphate, Dibasic {K1-M06} [Sigma-Aldrich, P3786-500G]
- 500uL V-bottom plate {K1-M07} [VWR, 89005-016]
- 96-Well skirted PCR plate {K1-M08} [Bio-Rad, MSP-9601]
- 1-Well Low Profile Reagent Reservoir {K1-M09} [Axygen, RES-SW1-LP]
- Agilent 96LT-180uL Tips {K1-M10} [Agilent, 19477.002]
- Axygen -80°C Rated Foil Seal {K1-M11} [Axygen, PCR-AS-200]

Assets

- Agilent LT-Bravo Automated Liquid Handling Platform with VWorks4 {K1-A01}
- Thermo Scientific Savant SC210A Concentrator {K1-A02}

Reagent Mixes

NB: Prepare 400nmol/uL NHS Propionate in Methanol {MIX05} immediately before use to prevent the solution from hydrolyzing(?).

ID	Name	Step	Composition	Volume/Well	Use
MIX01	500mM Sodium Phosphate, Monobasic	**K1**	119.98mg/mL NaH_2PO_4 {K1-M05} in HPLC-grade water {K1-M01}	N/A	Creation of MIX03.
MIX02	500mM Sodium Phosphate, Dibasic	**K1**	141.96mg/mL Na_2HPO_4 {K1-M06} in HPLC-grade water {K1-M01}	N/A	Creation of MIX03.
MIX03	500mM Sodium Phosphate Buffer, pH 8	**K1**	500mM Na_2HPO_4 in HPLC-grade water {K1-M01}	3uL	To keep histone samples at pH 7 during propionylation reaction.
MIX04	0.1% TFA	**K1**	0.1% TFA {K1-M03} in HPLC grade water {K1-M01}	~50mL	To quench the propionylation reaction.
MIX05	400nmol/uL NHS Propionate in Methanol	**K1**	68.4mg/mL of NHS propionate {K1-M04} in anhydrous methanol {K1-M02}	80uL	To derivatize lysine residues on histone proteins with propionyl groups.

Mix Preps and Mini-worksheets:

MIX01 – 500mM Sodium Phosphate, Monobasic

1. Weigh out at least 11.998g of sodium phosphate monobasic to make at least 200 mL
2. Calculate amount of water to add in mL by dividing amount weighed out by 00.0599
   - ❏ Amount weighed: _____ g
   - ❏ Divide by: 0.0599
   - ❏ Water to add _____ mL.

MIX02 – 500mM Sodium Phosphate, Dibasic

1. Weigh out at least 14.196g of sodium phosphate dibasic to make at least 200 mL
2. Calculate amount of water to add in mL by dividing amount weighed out by 0.07098

- ❑ Amount weighed: _____ g
- ❑ Divide by: 0.07098
- ❑ Water to add _____ mL

MIX03 – 500mM Sodium Phosphate Buffer, pH 8

1. Pipette 5.3mL of 500mM sodium phosphate monobasic {MIX01} into a 250mL bottle. ❑
2. Pipette 94.7mL of 500mM potassium phosphate dibasic {MIX02} into the bottle. ❑
3. Test the pH of the solution and ensure that it is at 8. ❑

MIX04 – 0.1% TFA

1. Measure 999 mL of HPLC-grade water {K1-M01} in a graduated cylinder and add to a 1L bottle. ❑
2. Pipette 1mL of TFA {K1-M03} into the bottle. ❑

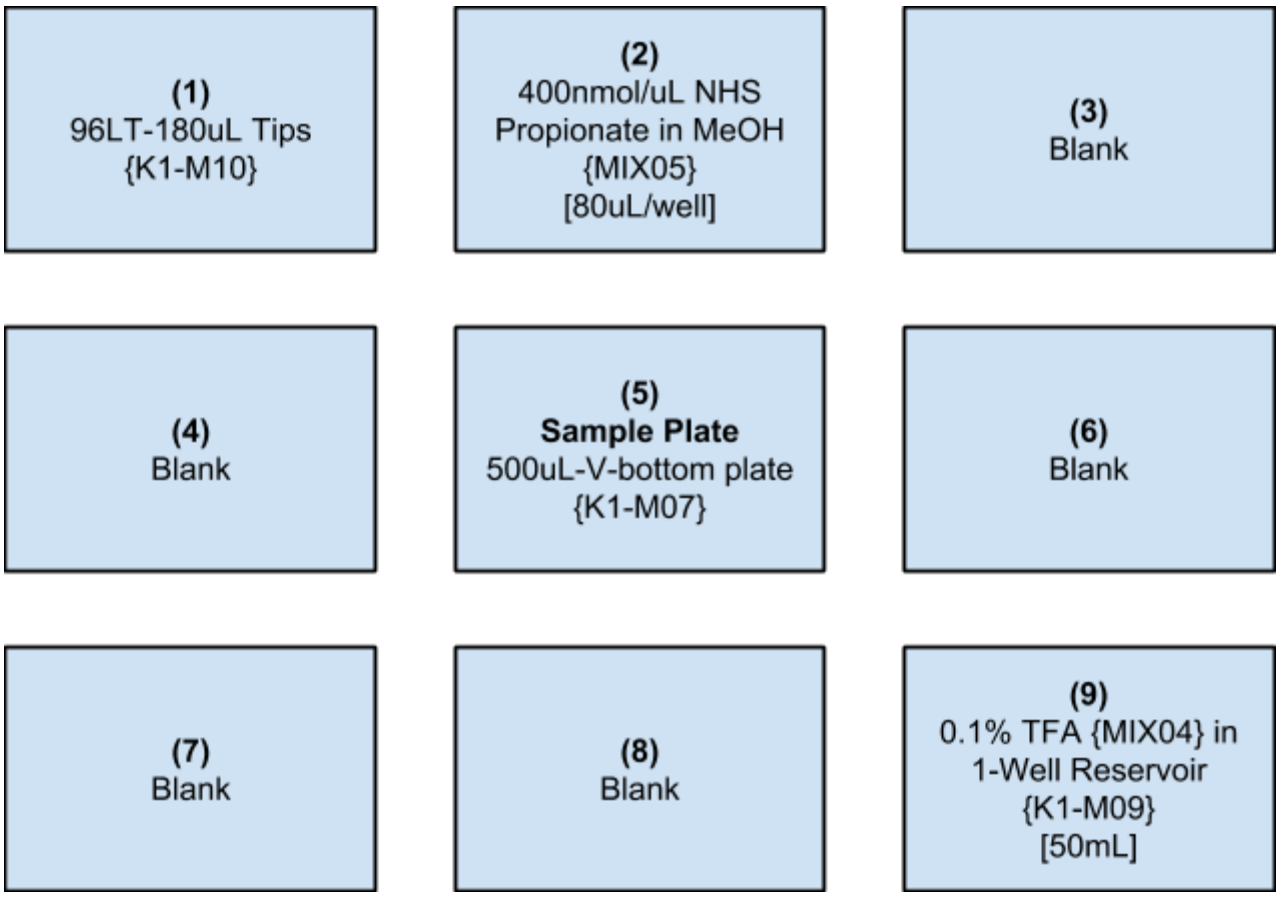
MIX05 – 400nmol/uL NHS Propionate in Methanol

3. Weigh out at least 684mg of NHS-propionate to make at least 10 mL
4. Calculate amount of water to add in mL by dividing amount weighed out by 68.4
   - ❑ Amount weighed: _____ mg
   - ❑ Divide by: 68.4
   - ❑ Methanol to add _____ mL.

Procedure

1. Acquire **500uL V-bottom plate** {K1-M07}. Label with study name, date, "Primary propionylation"
2. If using SILAC standardization, prepare the heavy standard triple mix
   2.1. Using the results from the Bradford Assay, combine an equal amount of histone from each of the three cell lines (HeLa, K562, 293T). Combine enough histone material so that each sample receives **5ug**. Add HPLC-grade water to the solution so that the concentration is an easy number like 5 ug/ul or 6 ug/ul
   2.2. Add **5ug of sample** to **5ug of heavy standard triple mix** in the assay plate.
3. If using heavy synthetic peptides for quantification, add **10ug of each sample** to the assay plate.
4. Adjust each sample to 100mM sodium phosphate buffer by adding 3uL of 500mM sodium phosphate buffer, pH 8 {MIX03}.
5. Adjust the total volume of each sample to **15uL** with HPLC-grade water {K1-M01}.
6. Fill a 1-well reagent reservoir {K1-M09} with approximately 50mL of 0.1%TFA {MIX04}.
7. Fill each well of a 96-Well skirted PCR plate {K1-M08} with 80uL of 400nmol/uL NHS Propionate in Methanol {MIX05}.
8. On the LT-BRAVO Marvin {A01} load the device file "Bravo with Heated Shakers.dev". This file is located at C:\\VWorks Workspace\Device Files\.

9. In the “Devices” page, click on “Agilent LT-BRAVO” and then “Marvin”. Select “Initialize all devices”.
10. Open the protocol file “PrimaryPropionylation_10ug_Protein.pro”. This file is located at · C:\\VWorks Workspace\Protocol Files\CF\Histones\.
11. Assemble the deck of the LT-BRAVO according to the following layout:



12. On the LT-BRAVO, toggle to “Simulation is on” at the top of the screen from “Simulation is off”.
    12.1. Press Start and the Run Configuration Wizard will pop up. Press Finish.
    12.2. A pop up entitled “Set Initial Values for Variables” will appear. Set the number of “Columns” to the appropriate amount of sample columns and press ok.
    12.3. The simulation will run and provide feedback on any warnings or errors that the protocol may encounter. If there are any unknown errors that come up, notify the key LT-BRAVO user and obtain help.
13. On the LT-BRAVO, toggle back to “Simulation is off”. Follow steps 12.1 and 12.3 to run the protocol.

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NB: The protocol will pause after the addition of NHS-propionate.
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14. After the protocol has paused, place a foil seal {K1-M11} on the sample plate and replace the plate in position 5 on the LT-BRAVO deck.
    14.1. Press GO.
    14.2. The LT-BRAVO will move the plate to the shaker where it will incubate at 25°C for 30 minutes with shaking at 800rpm.

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NB: After 30 minutes, the protocol will pause again. Remove the plate and spin it
down.
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15. Remove the foil cover and replace the plate in positon 4 at the heated shaker position.
    15.1. Press GO to continue the protocol.
    15.2. The LT-BRAVO will add 0.1% TFA to the sample plate and quench the reaction.
16. Clear the deck of the LT-BRAVO and move on to the **Oasis Desalt.**