7-1-15 BG

Propionic anhydride derivatization of Histones (solution)

OVERVIEW
2× propionylation
1× trypsin digestion
2× propionylation
Desalting (see Stage tip protocol)

PROTOCOL

1. Sample volume should be very low (~15-20 μL), preferably in deionized water. Dilute sample with equal volume acetonitrile (ACN). Add 0.5-1 μL of ammonium hydroxide to sample to make sure 7<pH <9. Check with pH paper.

2. Make propionic anhydride reagent by adding 25 μL of propionic anhydride to 75 μL of ACN (or in a 1:3 ratio of a suitable volume) **(make sure to seal propionic anhydride reagent bottle back under Argon gas afterwards; fill with Argon for about 30 seconds and be careful not to splash solvent to tube outlet of Argon tank. If so, wipe off outlet with kimwipe)**

3. Add about 1/4 volume of propionic anhydride reagent (relative to sample volume) to sample and vortex (pH goes down quickly to 4.0-6.0 as propionic acid is released). So if you started with around 30 μL of sample you would add ~8 μL of propionic anhydride reagent.

4. **Immediately** add NH_4OH, check to make sure pH ~ 8.0, then incubate @ RT for 20 minutes. Approximately half the volume of propionic acid reagent should be used to estimate the amount of NH_4OH to use. Therefore if you used 8 μL of propionic anhydride reagent, then add 4 μL of NH_4OH. However check pH and add more NH_4OH if necessary.

5. Dry down in speed vac to ~5 μL

6. Repeat steps 1-5 one additional time

7. Add 50-100 μL of ammonium bicarbonate (100 mM, pH =8.5) to sample to prepare for trypsin digestion. Check pH, it should be ~8.0, but if it still acidic, then add solid ammonium bicarbonate to sample by using pipet tip (ask for demonstration). After pH is ~8.0 add trypsin in a 1:10 protease:histone ratio and digest for 6-8 hours at 37 ^oC. The concentration of our trypsin is 0.5μg/μL, so you would need to add 10 μL of trypsin to a 50 μg sample.

8. Quench trypsin digestion by only freezing in -80^oC.

9. Vacuum speed vac sample down to ~5 μL, add 10 μL of deionized water, and then repeat steps 1-6 (thus two additional rounds of propionylation after the digestion).

10. Reduce sample volume for Stage tip (see stage tip protocol) to around 5 μL and reduce pH to ~3.0 by adding 0.1% acetic acid (~20 uL), and you may need to add 1 μL of glacial acetic acid to get pH down to 3.

Revision: July 16, 2016
Maintained by: Ben Garcia, Garcia Lab

Propionic anhydride derivatization of Histones (In Gel)

Day 1

In Gel Propionylation and Typsin Digestion

1. Cut out gel band and dice into small 1mm cubes.

2. Rinse with 50 μL of 100 mM NH_4HCO_3 and shake for 5 min

3. Remove NH_4HCO_3 buffer, add 100 μL of 100% acetonitrile and shake for 5 min

4. Remove acetonitrile and dehydrate in Vacuum centrifuge.

5. Make a 50/50 mix of propionic anhydride reagent and 100 mM NH_4HCO_3 (add 200 μL of propionic anhydride and 200 μL of 100 mM NH_4HCO_3).

6. Mix reagent vigorously on votex, as propionic anhydride and 100 mM NH_4HCO_3 do not mix well. After a vigorous mix, the reagent should look kind of cloudy, almost like an emulsion.

7. Add 100 μL of reagent immediately to dehydrated gel band and shake for 10 min

8. Remove excess reagent and rinse with 100 μL of 100 mM NH_4HCO_3 and shake for 5 min

9. Remove NH_4HCO_3 buffer, add 100 μL of 100% acetonitrile and shake for 5 min

10. Remove acetonitrile and dehydrate in Vacuum centrifuge.

11. Repeat steps 5-10 **twice** more (three rounds of in gel propionylation in total)

12. Dehydrated gel band is now ready for in-gel trypsin digestion, but could be stored dry for several days until you want to proceed with digestion.

13. Swell gel pieces in 12.5ng/ul trypsin (diluted in 100 mM NH_4HCO_3 and enough to cover gel pieces), on ice for 45 min (Trypsin stock: 500 ng/μL, add 20 μL stock to 780 μL 100mM NH_4HCO_3).

14. Remove supernatant and replace with minimal volume of the same 100mM NH_4HCO_3 buffer minus Trypsin, and allow to react for another 10 hours or overnight.

Day 2

Peptide Extraction

1. Rinse with 15 μL 50 mM NH_4HCO_3 pH 7.8 (15 min, shaking remove prior solution if there is a lot left)
2. Remove and keep rinse solution
3. Dehydrate with 20 μL of 50% MeCN 5% formic (15 min, drops pH to inactivate trypsin)
4. Collect and pool
5. Rehydrate with 10 μL NH_4HCO_3pH 7.8 (15 min, shaking)
6. Collect and pool
7. Dehydrate with 20 μL of 50% MeCN 5% formic. (15 min, shaking)
8. Collect supernatant and pool
9. Rehydrate with 10 μL NH_4HCO_3pH 7.8 (15 min, shaking)
10. Dehydrate with 100% MeCN (use excess about 3X of #9 volume, 15 min, don't remove supernatant)
11. Collect and Pool
12. Rehydrate with 10 μL NH_4HCO_3pH 7.8 (15 min, shaking)
13. Dehydrate with 100% MeCN (use excess about 3X of #9 volume, 15 min, don't remove supernatant)
14. Collect and pool
15. Reduce to near dryness (1-2 uL).
16. Perform two more rounds of in solution histone propionylation (see above protocol).
17. Reconstitute in 0.1% HOAc (minimum of 10 uL, better around 20-25 uL, sonicate tubes) and sample ready for STAGE Tip clean-up.

Revision: July 16, 2015
Maintained by: Ben Garcia, Garcia Lab