96 well S-trap Mini Plate
100 ug of sample digest in 50 ul buffer

To do before protocol:

- ☐ Make 50 mM TEAB (205 ul per sample + extra for trypsin)
- ☐ Make S-trap binding buffer (1.1 mL per sample)
- ☐ Make 100 ng/ul enolase (16 ul per sample)
- ☐ Make 250 mM TCEP (1.5 ul per sample)
- ☐ Defrost 500 mM DTT and 500 mM IAA (in dark; 6 ul per sample)
- ☐ Make 50% acetonitrile with 0.2% formic acid (80 ul per sample)
- ☐ Make methanol/chloroform 50%/50% (450 ul per sample)
- ☐ Find: 27.5% phosphoric acid (8.2 ul per sample), 250 unit/ul benzonase nuclease (0.5 ul per sample), 0.2% formic acid (80 ul per sample)
- ☐ Make 2% acetonitrile with 0.1% formic acid (50 ul per sample)
- ☐ Make batch control
- ☐ Label 2 batch of 1.5 mL tubes
- ☐ Heat block at 55C (tubes)
- ☐ Heat block at 47C (plate)
- ☐ S-trap plate map

1. pH of samples should be >7. Modify with 1M tris HCl 7.5 if necessary. Sample are in tubes.
2. Add 16 ul 100 ng/ul enolase.
3. Add 0.5 ul 250 unit/ul benzonase nuclease; mix by pipetting up and down several times.
4. Incubate samples at room temperature for 5 minutes. Vortex.
5. Reduce with 1.5 ul of 250 mM TCEP. Vortex.
6. Incubate at 55-60C for 15 minutes.
7. Let cool at room temp for 5 minutes.
8. Alkylate by adding 6 ul of 500 mM IAA. Vortex and incubate for 30 minutes, room temp, in the dark.
9. Spin tubes at 13,000 xg for 8 minutes to remove particulates.
10. Transfer liquid to new tube.
11. For sample volume of 74 ul, add 8.2 ul of 27.5% phosphoric acid. Vortex.
12. Test the pH of each sample. It should be <1. Add more phosphoric acid if necessary.
13. Add 350 ul S-trap binding buffer to lysate (6x volume of sample). Vortex.
14. Add up to 400 ul of acidified samples to the S-trap 96-well plate.
15. Spin the plate at 1500 x g for 2 minutes (all solution must pass through).
16. Wash columns 3x with 200 ul of S-trap binding buffer and discard flow-through.
17. In the hood, prep the methanol/chloroform. Add 150 ul to each plate well. Spin through at 1500 x g for 2 minutes.
18. Repeat step 17 2 more times.
19. Wash columns once with 150 ul S-trap binding buffer.
20. Centrifuge 1500xg for 2 minutes. Discard all flow-through

21. Put s-trap plate in a clean collection plate to collect peptides. ***All washes below will contain peptides and will be collected.***
22. Make trypsin and add to samples (4 ug in 125 ul per sample; see below)
23. Cap the s-trap plate loosely and incubate for 1 hour at 47C. Make sure some trypsin solution has percolated through column after 1 hour.
24. Add 80 ul 50 mM TEAB to columns. Centrifuge at 1500 xg for 2 minutes to elute peptides.
25. Add 80 ul 0.2% aqueous formic acid to each column. Centrifuge at 1500xg for 2 minutes.
26. Add 80 ul 50% acetonitrile with 0.2% formic acid. Centrifuge at 1500 xg for 2 minutes.
27. Pool all eluted peptide fractions into tubes.
28. Dry on speed vac (~2 hours)
29. Resuspend samples in 100 ul 2% acetonitrile with 0.1% formic acid.

36 samples

50 mM TEAB: 8 mL
1 M TEAB * x = 0.05 M * 8 mL
400 µl 1 M TEAB + 7.6 mL water
Check pH ~7.5

S-trap lysis buffer: 500 µl
1 M TEAB: 25 µl
20% SDS: 125 µl
1 M MgCl2: 1 µl
HALT: 5 µl
Water: 344 µl
pH ~8.5

100 ng/ul enolase: 600 µl
Aliquots are 400 ng/µl
400 ng/µl * x = 100 ng/µl * 600 µl
150 µl of enolase aliquot + 450 µl S-trap lysis buffer

S-trap binding buffer: 45 mL
1 M TEAB * x = 0.1 M TEAB * 45 mL
1 M TEAB: 4.5 mL
Methanol: 40.5 mL

250 mM TCEP: 60 µl
0.5 M * x = 0.25 M * 60 µl
0.5 M TCEP: 30 µl
Water: 30 µl

50% ACN + 0.2% formic acid: 3.5 mL

1.75 mL ACN
7 µl formic acid
1.743 mL water

Methanol/chloroform: 17 mL
8.5 mL methanol
8.5 mL chloroform