Systems Bioengineering Laboratory

Protein Isolation for Urinary Proteomics

Prepared by: Nanditha Anandakrishnan*
Version: 2.0
Date: 20-Oct-2020

Methods

1. Urine samples from COVID-19 positive patients are collected wrapped in a biohazard bag with a deidentified study label, immediately frozen on dry ice and then transferred to -80ºC until use.

2. Steps 4-20 are performed in a BSL2+ facility in adherence to the SOP, safety precautions and PPE requirements for COVID-19 (isolation gown, face shield, mask and double gloves).

3. On the day of protein precipitation, the sample is rapidly thawed at 37ºC or the samples can be thawed on ice overnight at 4ºC.

4. The Biosafety cabinet (BSC) surface is disinfected with Quatricide or any approved disinfectant, and a small autoclavable biohazard bag is placed for solid waste collection (includes everything that comes into the BSC). Do not dispose anything into the red biohazard bin.

5. The biohazard bag containing urine cups is wiped with Quatricide before moving it into the biosafety cabinet and the urine cups are removed from the biohazard bag.

6. A volume of 4-10 mL urine from the urine cup is transferred to a 15- or 50-mL conical tube using a 5- or 10-mL serological pipette. Remaining urine is aliquoted and stored in -80°C. Used pipettes are disposed into the solid waste collection bag.

7. Centrifuge buckets are removed from the tabletop centrifuge along with the adapter and containment lid and disinfected before moving it into the BSC.

8. The conical tube containing urine is transferred to the centrifuge bucket after decontamination and centrifuged at 2,000 rpm for 10 minutes at 4ºC.

9. Centrifuge buckets containing the samples are removed from the tabletop centrifuge are disinfected and moved into the BSC.

10. The tube is removed from the centrifuge bucket, decontaminated and the supernatant is transferred to a new 50 mL conical tube using a 5- or 10-mL serological pipette.

11. The centrifuge bucket, adapter and containment lid are thoroughly wiped down and inserted back into the tabletop centrifuge after decontaminating the rotor and centrifuge chamber walls.

12. For precipitation, 1 mL of urine supernatant is mixed with 8 parts ice-cold acetone (Sigma Cat. No. 179124) and one-part 1g/mL Trichloroacetic acid (Sigma Cat. No. 91228).

13. The solution is mixed by inverting tube a few times and incubated at -20ºC for 1 hour.

* Originally adapted from *Cornell University Proteomics and Mass Spectrometry Facility*

14. In the BSC, the micropipettes, tip box and markers are disinfected. The cabinet surface is wiped down and all used paper towels are disposed in the solid waste collection bag.

15. The solid waste is double bagged and secured with an autoclave tape (do not twist the autoclave bag to allow steam escape).

16. Disinfect the biosafety cabinet again including the walls and interior of the glass stash.

17. Place another small biohazard bag and dispose used paper towels into that and perform glove hygiene by spraying outer glove with Quatricide.

18. Put on new outer gloves and secure biohazard bag before disposing into the autoclavable waste accumulation area.

19. All subsequent steps of urine protein precipitation can be performed outside a BSL2+ facility.

20. After 1-hour incubation, the samples are centrifuged at 3,300 rpm for 60 minutes at 4ºC.

21. The supernatant is collected in a plastic beaker with an appropriate disinfectant before disposing down the drain.

22. The pellet is resuspended in 1 mL ice cold acetone using a 1 mL micropipette tip and centrifuged at 11,500 rpm for 15 minutes.

23. Step 22 is repeated twice to remove TCA completely and the pellet is allowed to air dry for 10 minutes.

24. The pellet is resuspended in 100 µL of rehydration buffer (7 M urea (Sigma Cat No. U5378), 2 M thiourea (Sigma Cat. No. T8656, 1% CHAPS (Thermofisher Cat. No. 28300) and 50 mM DTT (Thermofisher Cat. No. R0861) using a 100 µL volume micropipette tip.

25. The sample is vortexed if needed to completely resuspend the pellet.

26. Protein quantification is carried out using Bradford assay (Sigma Cat. No. B6916) against an albumin standard.

27. The protein is aliquoted (40 µg protein), flash frozen and stored at -80ºC until further use.

28. If using immediately, 30 µg of protein is mixed with LDS sample buffer (Life Technologies Cat. No. B0007) and run on 4-12% Bolt 4 to 12%, Bis-Tris mini protein gels (Life Technologies Cat. No. NW4122BOX) at 200 V for 22 minutes.

29. The gels are fixed using 50% methanol (Fisher Scientific Cat. No. A412-4), 10% acetic acid (Fisher Scientific Cat. No. A38-212) in water for 1 hour and stained using Coomassie R-250 (Bio-Rad Cat. No. 1610436) staining solution for an hour.

30. The gels are de-stained overnight in 50% methanol, 10% acetic acid and stored in 5% acetic acid at 4ºC until ready to be shipped for proteomics.