SOP-K001

Preparation of Plasma Samples for SPARC – Tan Rats

Objective: To collect plasma from live rat experiments for storage at -20˚C for further hormone assays.

Items to take over to Pierce Hall:

1. Inhibitor cocktail
    a. DPPIV – 10µl
    b. Protease inhibitor cocktail 50x – 20µl
    c. Aprotonin – 50µl
    d. Pefabloc – 10µl
    e. K3-EDTA – 10µl
    f. Mix above in blue Epindorf tube and centrifuge to spin contents down to bottom, keep cold.

2. 3 sets of strip tubes and labels (ZT-xxxxx S1, ZT-xxxxx S3, ZT-xxxxx S5)
3. Freezer rack

Source of Reagents

- DPPIV:
- Protease inhibitor cocktail:
- Aprotonin:
- Pefabloc:
- K3-EDTA:

Procedure: at Pierce Hall, room xxxxxxx

1. Turn on the Hettich refrigerated centrifuge so it can begin to cool down to 4 deg C.
2. Add 15µl of inhibitor coctail to each of 5 tubes for the Culex and firmly place cap making sure it is on good and level. (these are plastic reusable labels from BASI inc)
3. Place the plastic numbers (re-usable)1-5 one on each Culex tube, place up from bottom of tube.
4. Place the Culex tubes on the Culex machine in slots 1-5.
5. Put the labels on the strip tubes if they are not already.
6. When the first sample comes off the Culex, centrifuge on program #3 (4˚C, 10 minutes, 3,000rpm (?RCF) immediately.
7. Using the P200 pipette set at 85µl carefully try to remove as much of the plasma as possible and transfer to the appropriate pcr strip tube.
8. If needed switch to the P100 set to 15µl and remove remaining plasma without disturbing the red cell layer.
9. Keep the strip tubes in the freezer block (ISO Freeze) during the entire collection process.
10. Do this for each sample as it comes off the Culex machine, S1, S2, S3, S4, S5.
11. Place an orange dot on the label on top of all the tubes that have been collected using the orange sharpie. This just indicates the original tube from which the aliquots were taken.

12. Once the fifth sample has been collected, change the rotor on the Hettich centrifuge to the one for strip tubes.
13. Change the program to program #2, which is 6,000 rpm (??RCF), 10 minutes, 4˚C.
14. You will need to close the lid and press start and wait. It will recognize the rotor has been changed and will automatically STOP.
15. Once it is stopped open the lid again. Then close the lid and press START. It should now run through the program.
16. When stopped, remove the strip tube.
17. Using the P200 set to 25µl and the P100 and make two aliquots of 25µl for every sample. If any aliquot is short, write an "S" on the cap of that aliquot.
18. Once all samples have been aliquoted, spin them again on program #2 to get all the sample to the bottom.
19. Samples must be transported using the ISO Freeze sample rack to keep temperature at 4˚C
20. Make sure all lids are on tight, and place samples in -20˚C freezer. Samples are added over time to the next plate to be run and placed in a labeled Ziploc bag in the -20˚C freezer.
21. Samples must then be logged into the next platemap using the MPLEX software. Print a copy after each sample set has been added to the plate map for recording purposes.

Created by: Kathy Ragheb

Verified by:_____Date:_____ _____

Print Name Sign Name