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SPARC - Preparation of Plasma Samples from Rats

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1 Works for me Share

dx.doi.org/10.17504/protocols.io.261geodpol47/v1

SPARC
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ABSTRACT

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

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KEYWORDS

rat plasma collection

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31106

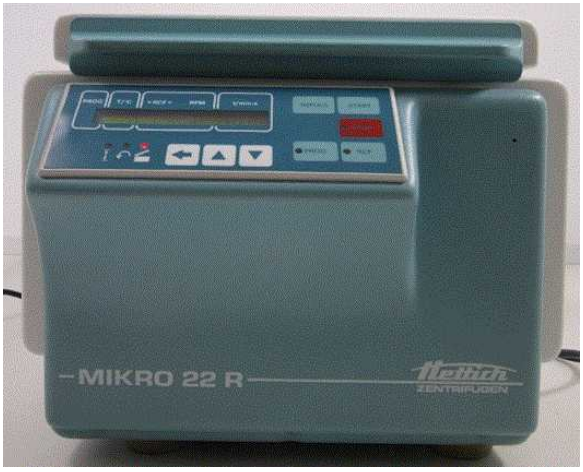
PARENT PROTOCOLS

In steps of

Quantification of the effect of gastric electrical stimulation location on circulating blood hormone levels

Quantification of the effect of gastric electrical stimulation location on circulating blood hormone levels

1 Turn on the Hettich Mikro 22R refrigerated centrifuge so it can begin to cool down to **4 °C** .



Hettich Mikro 22R refrigerated centrifuge (Cat # 1110, https://www.hettweb.com/)

Hettich Mikro 22R refrigerated centrifuge

Centrifuge

Hettich **Cat # 1110**

2 Add **15 µL** of Protease Inhibitor Cocktail to each of 5 Culex tubes (Vial Clear 8mm Crimp Rnd Bottom
300 µl Lot:0000071223 MicroLiter Wheaton Company)

Protease Inbibitor Cocktail **Sigma**

Aldrich Catalog #P2714-1BTL

Firmly place cap (Snap Cap 8mm, Natural with Cut T/S Septa Lot 0000000308 MicroLiter Wheaton Company) making sure it is on good and level. (these are plastic reusable labels from

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BASI inc)

3 Place the plastic numbers (re-usable-do not dispose of these number labels)1-5 one on each Culex tube, place up from bottom of tube.

Culex Automated Blood Collection System
Blood Collection
Culex **NxT**

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.

Labels
Dymo **Cat# 1750283**

6 When the first sample comes off the Culex, centrifuge on program #3 (4˚C, 10 minutes, **1000 x g** (**3.000 rpm**) **1000 x g** immediately.

7 Using the P100 pipette set at **85 µL** I carefully try to remove as much of the plasma as possible and transfer to the appropriate pcr strip tube. If needed set to **15 µL** and remove remaining plasma without disturbing the red cell layer.

Pipettman **Cat#75874-574**

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8 If needed switch to the P100 set to **15 µL** 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.

IsoFREEZE PCR Tube Chiller Rack
Freezer container
RPI 248002

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 Mikro 22R centrifuge to the one for strip tubes.

13 Change the program to program #2, which is **6.000 rpm** (RCR 4020)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.

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17 Using the P200 set to **25 µL** and the P100 and make two aliquots of **25 µL** 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 **25 µL** 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 plate map using the MPLEX software. Print a copy after each sample set has been added to the plate map for recording purposes. The purpose of adding each sample to the Plate Map for the MPLEX software is to maintain a fully digitized record for each assay.

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