Serum Assay Protocol

Razani Lab

V2 10-3-16

**Serum Collection**

-Fast mice for 5 hours (typically 8am to 1pm)

-Use capillary tubes to bleed mice from tail vein, 1 to 2 heparizined capillary tubes is sufficient depending on which assays need to be run. Typically 1 is plenty for glucose/FFA/trigs/chol, and more is required for ELISA assays such as IL-1β. Transfer blood to 1.7 mL microcentrifuge tubes sitting on ice.

-Spin all blood at 4C, 10 mins, 9600G (10k rpm on Sorvall Legend Micro 21R tabletop centrifuge)

-Carefully collect serum supernatant and transfer to labeled microcentrifuge tube, freeze at -80 until assay.

**Serum Measurement:**

**General notes:**

Thaw serum sample on ice – can make aliquot of 15 uL if all serum assays are run within a few hours and refreeze original tube. Free fatty acids and triglycerides are likely the least stable and should be run first.

-Crucial to thoroughly vortex samples before pipetting, mix reagent and sample very well (pipette mix), and to minimize and/or consider the impact of hemolysis (read a secondary wavelength).

-Generally it is most important to run all data that will be directly compared within one plate. This includes all groups of mice and all time points. Fitting more Biological replicates (more mice and using all time points) takes precedence over including technical replicates.

-Add 5 uL water to all wells prior to sample or standard addition; this allows sufficient time to pipette large groups of samples without the concern of overdrying/evaporation.

**Free Fatty Acids**

Kit: Wako diagnostics: <http://www.wakodiagnostics.com/r_nefa.html>

-Standard curve: 0, .15, 0.5, 1, 1.5 meQ/L

-Add 1 uL serum or standard to all wells

-Add 50 uL reagent A to all wells

-Incubate 15’ room temp

-Add 25 uL reagent B to all wells

-Incubate 15’ room temp.

-Read plate at 560 nm (primary), 670 nm (secondary)

**Triglycerides**

Kit: Thermo scientific Infinity Triglycerides Liquid Stable Reagent:

<https://www.fishersci.com/shop/products/thermo-scientific-triglycerides-reagent-infinity-triglycerides-liquid-2-x-125ml/tr22421>

-Standard Curve: 0, 12.5, 25, 50, 100, 200 mg/dL (1 mmol/L = 88.5 mg/dL)

-Add 1 uL of standards or samples, then 100 uL reagent to all wells

-Incubate 30 mins

-Read at 540 nm (primary) and 660 nm (Secondary)

**Cholesterol**

Kit: Thermo scientific infinity cholesterol : <https://www.fishersci.com/shop/products/thermo-scientific-total-cholesterol-reagents-infinity-total-cholesterol-2-x-125ml/tr13421>

1 mmol cholesterol = 38.67 mg/dL

- Standard curve: 0, 50, 100, 200, 500 mg/dL

**Dilute serum from ApoE -/- mice on atherogenic diets 4fold.**

-Add 1 uL of standards or samples, then 100 uL reagent to all wells

-Incubate 30 mins room temp.

-Read at 540 nm (primary) and 660 nm (Secondary)

**Glucose**

Kit: Thermo scientific infinity glucose hexokinase reagent: <https://www.thermofisher.com/order/catalog/product/TR15003>

-Standard curve: 0, 100, 200, 300, 400 mg/dL

-Add 1 uL of standards or samples, then 100 uL reagent to all wells

-incubate 30 mins room temp

-Read plate at 340 nm (primary) and 380 nm (secondary)