

Sample Collection:

Proper sample collection is critical to achieving valid results. Each investigator is responsible for his or her collection. If in doubt, please contact the core.

Depending upon the specific protocol, a volume of whole blood is usually collected in an EDTA coated tube and centrifuged for 15 minutes at 4°C. Care must be used if heparin is used instead of EDTA as an anticoagulant, since an excess will provide falsely high values in some assays. Use no more than 10 IU heparin per ml of blood collected.

Samples with gross hemolysis or lipemia may yield false results.

The plasma (compared to serum, plasma is cleaner, much easier to work with, and gives better results) is aliquoted into the appropriate labeled tube for the core (use a separate tube for each assay): for canine and human use 7 ml 12 x75 mm (e.g. Sarstedt tube #55.526) with push caps or for mouse use 0.5 ml 30 x 7.8 mm (e.g. Sarstedt tube #72.699) microfuge tubes.

A preservative must be added to the following samples (instructions for preparing these solutions can be found at the bottom of this page):

- Active Ghrelin: HCL-PMSF
- Catecholamines: EGTA-glutathione
- C-peptide: Trasylol (aprotinin)
- GLP-1: DDP-IV inhibitor
- Glucagon: Trasylol (aprotinin)

Active Ghrelin Pefabloc SC (AEBSF) Solution (or use HCL/PMSF as an alternative)

- The active form of ghrelin is very unstable and labile in serum/plasma due to the nature of the octanoyl group on serine-3. Samples should be processed as quickly as possible and kept on ice to retard the breakdown of active ghrelin.
- We recommend adding to Pefabloc SC (AEBSF) - Sigma Cat # 76307 to the blood upon collection.
- Care must be taken when using heparin as an anticoagulant, since excess will provide falsely high values. EDTA is recommended - use no more than 10 IU heparin per ml of blood collected.
- Specimens can be stored at 4°C if they will be tested within 4 hours. For longer storage, specimens should be aliquot and stored at -20°C or below. Multiple freeze/thaw cycles should be avoided.
- Avoid using samples with gross hemolysis or lipemia.

Catecholamine EGTA-Glutathione Solution

- 4.5 g EGTA (for 50 ml solution)
- 3.0 g Glutathione (for 50 ml solution)
- The solution must be titrated with NaOH to pH of 6.0 -7.4
- Use two beakers to prepare:
 - Beaker #1 - Measure EGTA and Glutathione and place in 50 ml beaker
 - Beaker #2 - in a small 15 ml glass or plastic beaker, measure 5 ml of 10N NaOH solution and fill to 15 ml with di-H₂O
 - Beaker #1 - Bring volume of solution to 30 ml with Di-H₂O (solution will be very cloudy) - place a stir bar in the solution and place on magnetic stirrer
 - Place pH probe in beaker # 1 and stir solution slowly while titrating.
 - Add NaOH solution from beaker #2 with a dropper while stirring. pH to 6.0-7.4
 - Use a funnel to pour in to a 50 ml calibrated flask and bring up to 50 ml volume with Di-H₂O.
 - Pour solution into dark glass bottle and keep in refrigerator. (Must be kept in a dark place, so do not leave out on counter too long).

- Add 20 μ l of EGTA-Glutathione per 1 ml of whole blood (dog/human); add 2 μ l per 100 μ l of whole blood (mouse/rat)
- Mix, Spin, Remove plasma for analysis
- Glutathione and EGTA are purchased from Sigma Aldrich
 - Catalog numbers Glutathione G4251 and EGTA E4378

C-peptide / Glucagon Trasylol (aprotinin) Solution

- Vanderbilt users can purchase Trasylol from the core. Aprotinin concentrate (~200,000 KIU/ml, PentaPharm) can be ordered from Centerchem.
- Dilute with water to 20,000 KIU and of that add 50 μ l per ml plasma or blood to give a final concentration of 1000 KIU per ml.

DPP-IV Inhibitor

- DPP-IV inhibitors are [commercially available](#)

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