

Procedure 3.1 – Hauenstein Parkinson’s Center Sample Collection

Date: _____ Technician: _____ Experiment ID: 3.1.55555mmdd

	Draw Time: _____		Draw Time: _____		Draw Time: _____
	Trizol Time: _____		Trizol Time: _____		Trizol Time: _____
	Draw Time: _____		Draw Time: _____		Draw Time: _____
	Trizol Time: _____		Trizol Time: _____		Trizol Time: _____

1. Prepare the following for each sample.

- 1- 125ml Nalgene bottle with 25ml 1X PBS (pH 7.4 w/o Calcium and Magnesium).
- 2- Leucosep tubes containing 15 ml Ficoll-Paque. (Centrifuge for 1 minute at 1000rpm).
- 1- 50ml conical tube containing 20ml Trizol.

*Perform steps 2-10 without interruption.
 The time from blood draw to cell lysis must be under 60 minutes.*

2. Collect 4 10mL blood samples in purple top EDTA Vacutainers, 1 10 mL blood sample in red top serum Vacutainer, and 1 urine sample in 120 mL collection cup.

3. Place urine sample on ice.

4. Immediately place 5 spots in 65 µL volumes of blood from the 10 mL red top serum Vacutainer onto the blood spot card. Allow blood spot card to dry.

5. Combine 4 purple top EDTA tubes in 125 mL bottle containing PBS.

6. Transfer equal volumes of diluted blood solution into 2 Leucosep tubes.

7. Centrifuge for 15 minutes at 1,000 g (2300 RPM) and 4°C in a swinging bucket centrifuge with brake disabled.

8. Collect 6.5 mL of plasma from the top of each Leucosep tube, pool in 50 mL conical centrifuge tube, and put on ice.

9. Collect the buffy coat with sterile filter pipettor. Transfer immediately to TRIzol tube.

10. Vigorously shake the TRIzol tube to lyse leukocytes and immediately place on ice.

11. Place clotted serum Vacutainer on ice.

12. Place blood spot card in sample collection bag.

13. Transfer samples on ice to the Institute.