

Methods for Sample Collection and Shipment

Plasma Collection

For plasma collection please follow the SOP developed by the Early Detection Research Network (EDRN, NIHMS94453-supplement-2-1.pdf), except add an additional purification step to further deplete platelets as described below.

The procedure described by EDRN entails (1) Collecting blood in EDTA collection tubes such as e.g. BD vacutainers catalog # 366450, (2) mixing the blood by inverting 8-10x in the EDTA tubes, (3) storing the tubes at 4C for up to 4 hours (preferably < 30 minutes) prior to centrifugation, (4) centrifugating the tubes at 1000 x g at room temperature for 10 minutes, (5) removing the plasma from the upper 2/3 of the tubes after centrifugation while avoiding the lower buffy coat layer and (6) transferring to secondary tubes for long-term storage.

The additional purification step to remove residual platelets, recommended by Ocean Ridge Biosciences, is conducted as follows:

- (1) Transfer up to 1.4 ml of Plasma to a 1.5 ml microcentrifuge tube.
- (2) Centrifuge the plasma at 10,000 x g at 4°C for 10 minutes.
- (3) Being careful to avoid any pellet, transfer the supernatant plasma to a clean 1.5 ml centrifuge tube for long term storage.

After depletion of platelets the plasma can be immediately stored at -80C. Please ship a minimum of 1.2 ml of plasma per sample on dry ice.

Blood Collection

Whole blood samples should be collected by the client in PAXgene Blood RNA Tubes (Part# 762165, PreAnalytiX, Hombrechtikon, Switzerland). The recommended amount of blood per sample is 5 ml (two PAXgene tubes, 2.5 ml each). Follow the instructions provided in the PAXgene Blood RNA Tube circular (PAXgene-Blood-RNA-Circular-2009.pdf), with the options mentioned here. In brief:

- (1) Make sure PaxGene blood RNA tubes are properly labeled and equilibrated to 18-25C prior to use.
- (2) Fill at least one "discard tube" or other collection tube with the blood before filling a PAXgene tube in order that there is no dead volume from the blood collection set, and thus the PAXgene tube is fully filled with 2.5 ml of blood.
- (3) Allow at least 10 seconds to elapse while filling the PAXgene tube. Make sure the blood stops flowing to the tube prior to terminating the fill or moving on to the next tube.
- (4) Immediately after blood collection invert the tube 8-10 times to thoroughly mix the blood with the RNA stabilization reagent in the tube.
- (5) Equilibrate the filled tubes for 2-4 hours at room temperature prior to freezing.
- (6) Freeze the tubes at -20C for 24 hours and then transfer the tubes to a -80C refrigerator for long term storage.

Ship the frozen tubes to Ocean Ridge Biosciences enclosed in a dry ice package. For this purpose it is recommended to mount the tubes in a pre-frozen plastic tube rack. Place the rack in a Styrofoam box surrounded by a thin layer of foam sheet or bubble wrap and thoroughly surround the cushioned tube rack with ground dry ice. Use at least 3-4 kilograms of dry ice per rack.