GENERAL INSTRUCTIONS

PGDx Sample Collection Procedure

Tumor tissue samples may be provided as frozen tissue, FFPE blocks or slides, pleural effusion, xenografts, cells, or DNA. If pleural effusion, DNA, or cells are submitted, the percentage (%) tumor must also be provided. If a xenograft sample is submitted, a normal sample of the mouse strain must also be included.

A unique ID should be assigned to each sample delivered. General information relating to the specimens will be collected for administrative purposes. For each sample submitted, please complete a test requisition form and an Excel sheet with specimen details (provided). E-mail Excel spreadsheet to lab@personalgenome.com.

DNA

DNA should be quantified using a dsDNA-specific method such as the Qubit or PicoGreen method.

- At least 1-3 µg of DNA per sample should be submitted (minimum 50 ng) in no more than 100 µL
- All DNAs must have a minimum concentration of 5 ng/µL (higher concentrations are acceptable)
- All DNAs must have a total volume of at least 10 µL
- The DNA solution buffer must be 10 mM Tris/1 mM EDTA
- DNA must be pure, intact, and of high molecular weight (DNA from FFPE-derived tissue and plasma is acceptable)
- DNA must be free from contaminating nucleic acids from other individuals or species
- OD 260/280 must be between 1.8–2.0
- Samples must not be PCR-amplified
- A brief description of the DNA extraction protocol(s) that were used should be submitted along with your DNAs

Cells or Pleural Effusion

At least 1 million cells in PBS along with % of tumor composition. Alternatively cells may be fixed and paraffin embedded.

NORMAL SAMPLE COLLECTION

Saliva Collection

Using the Oragene-DNA kit, 2 milliliters of saliva is sufficient for our analysis. Samples can be stored for 1 year at room temperature. Saliva collection tubes should be labeled with a unique identifier and date and time of acquisition. Tube should be wrapped in an absorbent paper towel and packed in a foam container delivered overnight at room temperature. Watch a video tutorial.

Whole Blood Collection

A venous blood draw of between 5 and 10 milliliters of blood is sufficient for our analysis. Blood should be drawn into a K2EDTA lavender top tube and gently rocked back and forth to mix the blood and the preservatives. Do not spin blood tube and do not freeze the blood tube unless they will be stored for >1 month. Samples may be stored at 4°C for less than 1 month. Samples that are to be stored for more than 1 month should be frozen at -20°C first and then transferred to a -80°C freezer. Do not place the tubes in styrofoam holders for freezing. Tube should be wrapped in an absorbent paper towel and placed within a biohazard bag. Tube should be packed in a foam container delivered overnight at room temperature.

TUMOR SAMPLE COLLECTION

Frozen Tumor Tissue

At least 100 milligrams of tumor tissue in a sealed tube sent overnight on dry ice. If sending from international locations, a courier service should be used to ensure that samples remain frozen throughout transport.

Formalin-Fixed Paraffin Embedded Tissue

SLIDES:

- One H&E stained, cover-slipped slide
- At least 5-10 unstained slides:
  - Fresh cut and air dried
  - Unstained
  - Not cover-slipped
  - 5-10 micron thickness is optimal
  - On “+” charged slides
  - All slides must be labeled with at least one unique identifier
  - Place the slides in a plastic slide shipping container, in a mailer with bubble wrap/insulation
  - Analysis of less than 5-10 slides may be possible – please contact us to discuss

BLOCKS:

- Place block(s) in a plastic bag
- Place block(s) and one cold pack in a styrofoam container
- Place the styrofoam container in a box
- Securely close the box

EQUIPMENT AND SUPPLIES

- 2 x 10 ml K2EDTA Vacutainer, Becton Dickinson, Cat #366643 or equivalent (amount dependent on application)
- 2 ml screw-cap tubes, RPI Cat #144536
- 1.5 ml screw-cap tubes, RPI Cat #144534
- 50 ml tubes, Fisher
- Freezer storage boxes, Fischer
- Centrifuge, Sigma, 5k15C with rotor 11156 (swinging bucket) or equivalent
- Centrifuge, Beckman, Microfuge 18 or equivalent

OPTIMAL PROCEDURE

Step 1. Blood Draw

1. Obtain venous blood by any standard phlebotomy technique from a peripheral access point or from a central line by trained personnel into BD Vacutainer K2EDTA tubes.
   - For PlasmaSelect please obtain 10-20mL venous blood.
2. Invert tubes several times after collection.
3. Transport the tube immediately to the laboratory. Time between draw and processing optimally shouldn't exceed 1 hour.

Step 2. Prepare Plasma From EDTA Blood Purple Top Tubes

1. Upon arrival in the laboratory, spin tubes at 814 g (2000 rpm, Sigma) for 20 min at 4°C or room temperature.
2. Transfer plasma supernatant to a 50 ml tube without disturbing the cellular layer using a 2 or 10 ml pipette.
3. Aliquot 1.05 ml plasma from the large tube to 1.5 ml tubes. (Note: About 5 ml of the 10 ml of blood will be plasma so approximately 5 aliquots).
4. Spin tubes at 18,000 g (~14,000 rpm, Beckman) for 10 min at room temperature.
5. Transfer 1 ml of plasma from the 1.5 ml tube to a fresh 2 ml tube without disturbing the pellet.

Step 3. Labeling of Tubes

Tubes for collection are labeled as follows:

- Patient ID
- Aliquot Number, e.g. 1, 2, 3, etc.
- Date, e.g. July 01 2012
- Volume, e.g. 1 ml

Step 4. Storage

Plasma vials are stored at -80°C

Note: For research use only (RUO) plasma analyses: If unable to collect and send frozen plasma, K2EDTA can be used to collect blood. Store K2EDTA tubes on ice or in a refrigerator (ideally within 1 hour of blood draw). Ship tubes to PGDx for arrival within 1 day from time of blood draw (samples can be processed if received up to 3 days from the time of blood draw, but sensitivity may be reduced by ~25%). Tubes should be wrapped in absorbent paper towel and placed in a biohazard bag. K2EDTA tubes should be placed in a sturdy box with cold packs and shipped overnight to arrive at PGDX at 4ºC.

CONTACT

Sample Delivery Address:

Personal Genome Diagnostics
2809 Boston Street, Suite 503
Baltimore, MD 21224
Tel: (443) 602-8833

Sample Collection and Shipping:

lab@personalgenome.com
(443) 602-8833

Please send an email to lab@personalgenome.com to notify us that the sample has been sent.

Samples must be sent for arrival on Monday through Saturday.