Genfind v2

Extraction of gDNA from Tissue

Please reference the current Genfind v2 protocol for product information and a detailed description of use.

Protocol

1. In a 2 mL microcentrifuge tube homogenize up to 20 mg of tissue (up to 10 mg thymus and 5 mg spleen) in 400 µL of Genfind v2 Lysis Buffer plus 9 µL of Genfind v2 Proteinase K (96 mg/mL). See below for recommended homogenization procedures.
2. Transfer 400 µL of homogenized sample to the well of a 1.2 ml 96-well plate (Thermo Fisher AB1127). Seal the top of the plate with an adhesive PCR plate seal.
3. Incubate the plate in a 37°C water bath for 30 minutes.
4. Proceed to the remaining steps of the GenfindTM v2 Plate Purification Procedure for 200 µL of blood/serum from the binding step on.

Recommended Homogenization Methods

Complete homogenization of the tissue is a critical step in isolating high quality DNA. Incomplete homogenization may result in lower yields and decreased purity of the isolated DNA. Several protocols for the homogenization of various tissues are commercially available. Selection of the best method for a particular range of uses should be determined experimentally. The homogenization protocols and equipment listed below have been used successfully in our research laboratory.

- Keep tissue frozen on dry ice as much as possible to minimize degradation.
- Cut samples on dry ice to minimize degradation.
- Weigh tube without tissue and re-weigh with tissue to make sure that the appropriate amount of Lysis Buffer and Proteinase K are added to the sample.
- If foaming is a problem, perform the homogenization in a 15 mL or 50ml tube.
- If you have enough starting material, make up an extra sample to compensate for dead volume and the foaming that may occur during and post homogenization. For example if processing two tissues samples (40 mg) homogenize enough for 3 (60 mg).
- If using a roto- stator homogenizer, homogenize using an up and down motion in the tube.

Recommended equipment for homogenization

- For tube format: tissue dispersing device: IKA Ultra Turrax (http://www.ika.de/ika/home.html) using a 5 mm dispersing element or Brinkman Polytron homogenizer
- For 96 well plate format: Plate vortexer (Troemner VX2400 Multitube Vortexer) and two

- **3.2 mm stainless steel beads (http://www.biospec.com/ part number 11079132ss) per well. 2 ml deepwell plate (http://www.abgene.com/ Product # AB-0661); plate seal (http://www.abgene.com/ Product # AB-0580)**

In general tissues may be divided into three groups or types for the purpose of homogenization:

1. **soft tissues such as liver, kidney, and lung**
2. **fibrous tissues, such as skeletal, cardiac and vascular smooth muscle**
3. **lipid rich tissues, such as adipose and brain.**

Up to 20 mg of tissue may be homogenized in 400 µL of Lysis Buffer/ PK in a microcentrifuge tube. Larger amounts of tissue may be homogenized simply by using a 15ml/ 50ml conical tube and scaling up the of the Lysis Buffer and Proteinase K. Use an additional 400 µL of Lysis and 9 µL Proteinase K for every additional 20mg of tissue.

For volumes above 2 mL total, a larger tissue dispersing element should be used (8 to 10 mm) to ensure complete homogenization. Foaming of the sample during lysis can be minimized by keeping the dispersing element fully submerged in the liquid.

1. **Soft tissue, tube format: Samples should be homogenized at the highest speed setting for about 2 minutes.**
2. **Fibrous tissue, tube format: Samples should be homogenized at the highest speed setting for about 5 minutes. In addition the 37°C lysis incubation can be extended to 45 minutes for particularly tough tissue such as vascular smooth muscle.**
3. **Lipid Rich Tissues, tube format: Samples should be homogenized at the highest speed setting for about 30 – 90 seconds. Special care should be taken to avoid excessive foaming of lipid rich tissues during homogenization.**