E-mail: sales@bluegene.cc tech@bluegene.cc www.elisakit.cc www.bluegene.cc

Sample Preparation Guide

Serum - Use a serum separator tube and allow samples to clot for 2 hours at room temperature or overnight at 2-8°C. Centrifuge at approximately $1000 \times g$ (or 3000 rpm) for 15 minutes. Collect serum and assay immediately or aliquot and store samples at -20°C or -80°C.

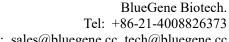
Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at $1000 \times g$ (or 3000 rpm) at 2 - 8°C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C or -80°C.

Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissues were rinsed in ice-cold PBS to remove excess blood thoroughly and weighed 500mg before homogenization. Minced the tissues to small pieces and homogenized them in 500ul of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm). Collect the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.

Cell lysates - Cells should be lysed according to the following directions.

- 1) Adherent cells should be detached with trypsin and then collected by centrifugation. Suspension cells can be collected by centrifugation directly.
- 2) Wash cells three times in PBS. As for the collection of the samples, the amount of cells should be no less than 10⁸ in 200ul PBS.
- 3) Cells were resuspended in PBS and subjected to ultrasonication for 3 times.

 Alternatively, freeze cells at -20 °C. Thaw cells with gentle mixing. Repeat the



BLUE GENE
FOR LIFE SCIENCE

E-mail: sales@bluegene.cc tech@bluegene.cc www.elisakit.cc www.bluegene.cc

freeze/thaw cycle for 3 times.

4) Centrifuge at 1000×g (or 3000 rpm) for 15 minutes at 2-8 °C to remove cellular debris.

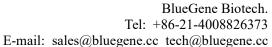
5) Assay immediately or store samples at -20°C or -80°C.

Cell culture supernatants and other body fluids - Centrifuge cell culture media at $1000 \times g$ (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20° C or -80° C.

Urine - Use a sterile container to collect urine samples (first urine of the day (mid-stream). Remove any particulates by centrifugation for 15 minutes at 1000× g, 2 - 8°C and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

Saliva - Collect 2-3 ml of saliva into a clean glass tube without force or inducement and before eating, drinking, or brushing the teeth. Simply rinse the mouth with water before collection. Do not use blood-contaminated specimens. Centrifuge samples at $1000 \times g$ (or 3000 rpm) for 15 minutes to remove debris. Assay immediately or store samples at -20° C or -80° C.

Fecal samples - Mince Fecal samples into small pieces and homogenize them in a certain amount of PBS with a glass homogenizer on ice. The resulting suspension was subjected to ultrasonication or to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifugated for 15 minutes at 1500×g (or 5000 rpm). Remove the supernate and assay immediately or aliquot and store samples at -20°C or -80°C.





E-mail: sales@bluegene.cc tech@bluegene.cc www.elisakit.cc www.bluegene.cc

Milk samples - Use a sterile container to collect milk samples. Then centrifuge for 15 minutes at 1000×g, 2 - 8°C and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.