GRS Genomic DNA Kit – Tissue

#GK03.0100 (100 preps) | GK03s (trial size, 4 preps)
(FOR RESEARCH ONLY)

SUGGESTED PROTOCOL FOR DNA PURIFICATION FROM BONE AND TOOTH

1) Cush the bone or teeth into small fragments (<5 cm) and grind to a fine powder by either:
   a) Place fragments in a mortar and pestle and pour in liquid nitrogen. Grind the bone/teeth while immersed in liquid nitrogen and keep pouring liquid nitrogen to ensure the sample remains immersed at all time.
   b) Place fragments in a metal blender half-filled with liquid nitrogen. Grind the bone/teeth while completely immersed in liquid nitrogen.

2) If sample is less than 100mg, transfer into a 1.5-ml microtube and proceed immediately to step 7.
   If sample is between 100mg and 5g, proceed with step 3.

3) Transfer 100mg – 5g to a 50-ml centrifuge tube and add 40 ml of 0.5M EDTA pH 7.5 and incubate with regular agitation (e.g., rotator) at 37ºC for 24 hours.

4) Centrifuge the sample at 2,000g for 15 minutes and discard the supernatant. Repeat steps 3 and 4 several times for complete decalcification. This process usually requires 3 to 5 days.

5) Wash the pellet with 40ml of sterile water. Centrifuge at 2,000g for 15 minutes, discard the supernatant and repeat this washing step three times.

6) Transfer up to 50mg into a 1.5ml microtube and proceed with step 7.

7) Add 360µl of Buffer BC2 and 40µl of Proteinase K (10mg/ml) and vortex for 30 seconds. Incubate at 60ºC until lysis is complete. During incubation, vortex occasionally to disperse the sample.

8) Add 400µl of Buffer TC1 and shake vigorously for 10 to 15 seconds.

9) Add 400µl of absolute ethanol and shake immediately for 10 to 15 seconds.

10) Place a gDNA plus mini column in a 2-ml collection tube and transfer 600 µl of the mixture to the column. Centrifuge at 14,000 to 16,000g for 1 minute. Discard the flow-through, place the column back in the collection tube and transfer all of the remaining sample mixture to the column.

11) Centrifuge at 14,000- 16,000g for 1 minute. Discard the collection tube containing the flow-through and place the column into a new 2-ml collection and proceed with the washing step of the normal tissue protocol.