

GRS Genomic DNA Kit – BroadRange

#GK06.0100 (100 preps) | GK06s (trial size, 4 preps)
(FOR RESEARCH ONLY)



SUGGESTED PROTOCOL FOR DNA PURIFICATION FROM INSECT TISSUE

- 1) Transfer up to 50mg of insect tissue to a mortar and add liquid nitrogen. Grind the tissue thoroughly using a pestle. If necessary, refill the mortar with liquid nitrogen in order to keep the sample frozen.
- 2) Transfer the powder to a 1.5-ml microcentrifuge tube and add 200µl of Buffer BR1 and 20µl of Proteinase K (10mg/ml) to the sample. Mix thoroughly by vortexing and incubate at 60°C for 1 to 3 hours (or until the lysate becomes clear). During incubation, invert the tube regularly or alternatively use an auto shaking system. [At this time, preheat the Elution Buffer in a 60°C water bath].
- 3) Centrifuge at 14,000g-16,000g for 2 minutes to remove any remaining insoluble debris. Transfer the cleared supernatant to a new 1.5-ml microcentrifuge tube and add 200µl of Buffer BR2. Mix by shaking vigorously and incubate at 60°C for 5 minutes.
- 4) **[optional; when RNA-free DNA is required]** Allow the mixture to cool to room temperature and add 5µl of RNase A (10mg/ml), vortex, and incubate for 5 minutes at room temperature.
- 5) Add 200µl of absolute ethanol to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.
- 6) Place the gDNA plus spin column in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.
- 7) Centrifuge at 14,000g-16,000g for 1 minute. If the mixture did not flow through the membrane completely, increase centrifugation time. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.
- 8) Add 400µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the gDNA plus spin column back in the collection tube. Add 600µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds (*Ensure ethanol was added 1st time prior to use).
- 9) Discard the flow-through and place the gDNA plus spin column back in the collection tube and centrifuge for another 3 minutes at 14,000g-16,000g to dry the matrix of the column.
- 10) Transfer the spin column to a new 1,5-ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3 to 5 minutes. Notes: Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5. Standard elution volume is 100µl. To increase concentration, elute with 30-50µl. To increase yield, elute with 200µl.
- 11) Centrifuge at 14.000g-16.000g for 1 minute to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20°C