GRS Genomic DNA Kit – Tissue

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

SUGGESTED PROTOCOL FOR DNA PURIFICATION FROM AMNIOTIC FLUID

1) Transfer approximately 10ml (up to 15ml) of amniotic fluid to a 15-ml centrifuge tube (not provided) and harvest the cells by centrifugation for 3 minutes at 14,000g-16,000g. Discard the supernatant.

2) Resuspend the pellet in 200µl of Buffer BC2 and transfer to a 1,5-ml microcentrifuge tube. Add 10µl of Proteinase K (10mg/ml), mix by shaking vigorously, and incubate at 60ºC for 30 minutes. During incubation, invert the tube regularly.

3) Add 200µl of Buffer TC1, mix by shaking vigorously, and incubate at 60ºC for at least 20 minutes. During incubation, invert the tube regularly. [At this time, preheat the Elution Buffer in a 60ºC water bath].

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 genomic DNA mini 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. 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 genomic DNA mini 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 genomic DNA mini 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 37ºC (incubator) for 10 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 for 1 minute at 14.000g-16.000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20ºC