

Circulating DNA isolation procedure

1. Take **0.5 ml** of sample and put it into a 1.5 ml microtube (provided)
Note: To avoid cellular contamination, it is important the way samples have been treated before storage or DNA isolation (see page 7).
When using less than 0.5 ml, reduce the volume of the kit solutions proportionally.
2. Add **100µl** of **Solution A** and **30 µl of proteinase K** and vortex gently
Note: Incubate solution A at 55°C and mix gently before addition to avoid salt precipitates.
3. Incubate at 55°C overnight.
4. Cool the samples from step 3 by incubating approximately 5 minutes on ice.
5. Add **200µl** of **Solution B** and vortex gently about 15 seconds.
6. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 10 minutes at room temperature.
7. Carefully pipet the supernatant into a new 1.5 ml microtube (provided), discarding the remaining pellet.
8. Add **80µl** of **Solution C** and **600µl** of **Solution D**
9. Shake slightly by inverting the tube several times until a homogenous solution is observed
10. Incubate the tube at room temperature for 10 minutes in a vertical position
11. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 5 minutes at room temperature
Note: Usually, a little pellet forms.
12. Discard the supernatant with care
13. Add **500µl** of **Solution E**
14. Centrifuge at full speed (20,000 x g; 14,000 rpm) for 5 minutes at room temperature.
Optional: An additional wash with 70% ethanol can be done to avoid salt excess in the final sample.
15. Discard the supernatant with care and place the tube (containing the pellet) open for 5-10 minutes to dry the pellet.
Note: Pellet is easily removable from the bottom of the tube, so supernatant must be discarded very carefully to avoid sample loss.
16. Add **10-50µl** of **Solution F** and pipet up and down carefully to re-suspend the purified DNA.
Note: Nuclease-free water can be used but is not recommended for long time storage.
17. Optional, incubate the tube at 37°C for 30 minutes to help DNA solubilisation
18. Use immediately or store at 4°C (if to be used during the next 48 hours) or at -20°C (for longer storage)