

Protocol Name: DNA Purple Top v1

Notes:

- Equilibriate samples to room temperature
- Ensure reagents have been prepared properly (see manual instructions on pages 14-15). Equilibriate reagents to room temperature
- Turn on BioOven to 70 °C for use in step 4
- If precipitate has formed in Buffer AL, dissolve by incubating at 56 °C

1. Pipet 400ul QIAGEN Protease into the bottom of a 15ml centrifuge tube (*Protease stored in -20 °C freezer*).
2. Add 4ml sample blood and mix briefly (*add appropriate volume of PBS if sample volume is less than 4ml*).
3. Add 4.8ml Buffer AL to the sample. Mix thoroughly by vortexing at least 3 times, for 5 seconds each time.
4. Incubate at 70 °C in BioOven for 10 minutes.
5. Add 4ml 100% ETOH to the sample, and mix again by vortexing.
6. Carefully transfer 3.3ml onto a QIAamp Midi column placed in a 15ml centrifugation tube. Avoid spilling and do not moisten the rim of the QIAamp Midi column. Close the cap and centrifuge for 3 minutes at 1881g/3000rpms, room temperature, brake on using a swing-out rotor with adapters for round-bottom tubes (*Do not overtighten caps. If caps are tightened until they snap they may loosen during centrifugation and damage the centrifuge*).
7. Remove the QIAamp Midi column, discard the filtrate, wipe off any spillage from the thread of the 15ml centrifugation tube before re-inserting the QIAamp Midi column, and place the QIAamp Midi column back into the 15ml centrifugation tube. Load another 3.3ml onto the QIAamp Midi column, close the cap, and centrifuge for 3 minutes at 1881g/3000rpms, room temperature, brake on.
8. Remove the QIAamp Midi column, discard the filtrate, wipe off any spillage from the thread of the 15ml centrifugation tube before re-inserting the QIAamp Midi column, and place the QIAamp Midi column back into the 15ml centrifugation tube. Load remaining sample onto the QIAamp Midi column, close the cap, and centrifuge for 3 minutes at 1881g/3000rpms, room temperature, brake on.
9. Remove the QIAamp Midi column, discard the filtrate, wipe off any spillage from the thread of the 15ml centrifugation tube before re-inserting the QIAamp Midi column, and place the QIAamp Midi column back into the 15ml centrifugation tube. Carefully, without moistening the rim, add 4ml buffer AW1 to the QIAamp Midi column. Close the cap and centrifuge for 5 minutes at 3345g/4000rpms, room temperature, brake on.
10. Remove the QIAamp Midi column, discard the filtrate, wipe off any spillage from the thread of the 15ml centrifugation tube before re-inserting the QIAamp Midi column, and place the QIAamp Midi column back into the 15ml centrifugation tube. Carefully, without moistening the rim, add 4ml of Buffer AW2 to the QIAamp Midi column. Close the cap and centrifuge for 25 minutes at 3345g/4000rpms, room temperature, brake on.

11. Wipe any spillage off the QIAamp Midi column and place the QIAamp Midi column in a clean 15ml centrifugation tube and discard the tube containing the filtrate. Add 600ul of Buffer AE, close the cap, and incubate at room temperature for 5 minutes. Centrifuge at 3345g/4000rpms for 10 minutes, room temperature, brake on.
12. Reload the 600ul of eluate containing the DNA onto the membrane of the QIAamp Midi column. Close the cap and incubate at room temperature for 5 minutes. Centrifuge at 3345g/4000rpms for 10 minutes, room temperature, brake on.
13. Quantify on Nanodrop.