Nucleic Acids isolation procedure

1. Transfer up to 150 µl of sample in a 1.5 ml microcentrifuge tube (liquid samples, e.g. blood, plasma, urine, saliva, swaps transport medium…) or directly transfer the solid sample (tissue, biopsy, cell pellet…) in a 1.5 ml microcentrifuge tube.
2. Add **Solution A** up to 450 µl and vortex gently. For solid samples, add directly 450 µl of Solution A. For liquid samples, add the required volume to reach 450 µl (e.g. 150 µl sample and 300 µl Solution A, 25 µl sample and 425 µl Solution A, etc.)

   Note: *Mix gently before addition if Solution A was stored at 4°C*
3. Incubate at 56°C for 20 minutes. Use of a horizontal shaker (at 100-150 rpm) is optional but preferable.

   Optional RNase treatment: *heat sample at 70°C for 5 minutes. Add RNase (not provided) 100 µg/ml (final concentration) and incubate at 37°C for 5 minutes. Finally, continue to step4.*
4. Cool the samples (from step 3) by incubating approximately 5 minutes on ice or a refrigerator.
5. Add 150 µl of **Solution B** and vortex gently.
6. Centrifuge at 17000 g (approx. 13500 rpm) for 10 minutes, at room temperature.
7. Carefully pipet the supernatant into a provided 1.5 ml microcentrifuge tube, discarding the remaining pellet.
8. Add 60 µl of **Solution C** and 700 µl of **Solution D**.
9. Shake slightly by inverting the tube several times until a homogenous solution is observed.
10. Incubate the tube at -20°C for at least 10 minutes in a vertical position.

    Note: *Sample can be stored at this point if you need to postpone the procedure.*
11. Centrifuge at 17000 g (13500 rpm) for 10 minutes at room temperature.

    Note: *Usually, a little pellet forms.*
12. Discard the supernatant with care.
13. Add 500 µl of **Solution E**.
14. Centrifuge at 17000 g (13500 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.

    (*)Pellet is easily removable from the bottom of the tube, so supernatant must be discarded very carefully to avoid sample loss.
16. Add 30-50 µl of **Solution F** and pipet up and down carefully to resuspend the pellet.

    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 solubilisation.
18. Use immediately or store at 4°C (if used during the next 48 hours) or at -20°C (for longer storage).