Protocol for Human DNA extraction from STABILIZED stool samples into DANASTOOL Sample Collection Kit

1. Transfer **1.0 ml of stabilized stool sample** to a 1.5 ml microtube.

Before transferring the sample, make sure that the sample is completely homogenized, for the transfer it is recommended to cut a 1000 µl tip to make the mouth wider and take 2 x 500 µl, mixing the sample well with the micropipette.

2. Incubate the sample for **10 minutes at 55ºC** in a thermal mixer under continuous shaking at 900 rpm.

3. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **600 µL of the supernatant** to a clean microcentrifuge tube.
   IMPORTANT: A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.

4. Add **200 µl EC Buffer Vortex.** Incubate at 4ºC for 5 minutes

5. **Centrifuge at 14.000 rpm for 5 minutes**. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

6. **Add 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.**

7. Add **250 µl of Binding Buffer** and vortex briefly.

8. Add the lysate into reservoir of a combined Microbial DNA Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

9. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer. Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

10. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

11. **Dry silica membrane.** Centrifuge at 14.000 rpm for 3 minutes.

12. Place the Microbial DNA Column into a 1.5 ml nuclease-free tube (not provided) and add **200 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

13. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The purified DNA is in the tube.