Rumen content DNA extraction with PowerSoil kit

Method for rumen digesta (**Liquid Phase**):

1. Take 250µl of liquid phase and put them in the PowerSoil tube
2. Follow the kit protocol
3. In the last step (elution) use 50µl 70°C DEPC water, instead of solution C6; centrifuge 10.000 rpm, 1 min.

Method for rumen digesta (**Solid Phase**) (modified from Kong et al. 2010):

1. Put 250mg of solid phase in **lysis A tubes** (use pipette tips)
2. Add 750µl of 0.4M **potassium phosphate** buffer
3. **Bead beat** 3x30sec, speed: 4.5 until digesta is homogenized (always keep samples on ice when using bead beater)
4. **Centrifuge** samples 10 mins at 10,000rpm
5. Discard liquid and add 500µl **Pre-lysis buffer** (20mM Tris/Cl, 2mM EDTA, 1% Triton-X 100; pH 8), stir and vortex until pellet is resuspended (up&down with pipette)
6. heat samples at **95°C** for 5min on thermoblock, slight shaking (900min-1), turn thermoblock down to 70°C afterwards (it takes around 10mins to lower the temperature)
7. **centrifuge** at 14,000 rpm for 5min
8. separate **supernatant** (ca. 600µl) in 2ml Eppi – store on ice until use with Kit
9. add 1.2ml of 0.4M **potassium phosphate** and resuspend pellet (stir with pipette tip after adding the first 600µl, then add with fresh pipette tip second 600µl)
10. add 100μl of 100mg/ml **lysozyme** and 10μl of 2.5U/μl **mutanolysin**
11. **Vortex** until pellet is resuspended completely
12. **incubate** at 37°C for 30min in incubator, shake at 300mov/min
13. add 20μl of 20mg/ml **Proteinase K**
14. **incubate** for 1h at 56°C, in incubator, shake at 300mov/min \*\*
15. place in **bead beater** 3x45 sec, speed: 4.5
16. **centrifuge** at 14.000rpm, 3min
17. Pool the **supernatant** (ca 1300µl) with the other supernatant from step 7
18. Sample is ready to be processed with Power Soil Kit; use 250µl of sample.

POWERSOIL KIT PROTOCOL MODIFICATION:

* instead of 10 min Vortex adapter only incubate for 1 min on the vortex adapter
* elute DNA with 50µl 70°C DEPC water, centrifuge 10.000 rpm, 1 min.

Store samples at -20°C.

\*\*Samples can be stored on ice for up to an hour

**The enzyme order numbers are:**

Bei Sigma Aldrich

|  |  |
| --- | --- |
| KatNr. | Artikel |
| L1667 | Lyzosyme (powder) |
| M9901 | Mutalolysin (powder) |
| 3115887001 | Protienase K (solution) (PCR Grade) |

**Potassium Phosphate Buffer:**

|  |  |  |
| --- | --- | --- |
| Desired pH | 1M K2HPO4 | 1M KH2PO4 |
| 6.8 | 248,5ml | 251,5ml |

1. Make 1M concentrations of K2HPO4 and KH2PO4 with ddH20 (weight amount of chemical that is given on bottle, plus add ddH2O up to 1 liter!)
2. Combine in above ratio in a new bottle
3. Mix 500mL 1M Potassium Phosphate buffer with 750mL ddH20
4. Add HCl/ NaOH to bring the pH back to 6.8 with continual stirring

**Pre-Lysis buffer:**

1. 1ml 2M Tris/HCl
2. 400µl 0.5M EDTA
3. 1ml Triton X-100
4. fill up to 100ml with ddH2O (97,6ml) 🡪 pH 8 (with HCl and NaOH)

**Mutanolysin:**

Powder 5000U. Desired concentration: 2,5U. Dilute the powder with 2ml ddH2O.

Things needed for 200 samples:

* **Lysis A tubes** (200)
* **Potassium phosphate buffer** **0,4M** (150ml + 240ml)

1. 1M K2HPO4
2. 1M KH2PO4
3. ddH2O

* **Pre-Lysis buffer** (100ml)

1. 20mM Tris/Cl
2. 2mM EDTA
3. 1% Triton-X 100

* **Lysozyme 100mg/ml** (2.0g; 20ml)
* **Mutanolysin 2,5U/µl** (5000 U) (2ml)
* **Proteinase K 20mg/ml** (4ml)
* **Power Soil DNA Isolation Kit** (MoBio) (200 preps)