Protocol 005
RNA isolation of *Clostridium acetobutylicum* (Oelmüller *et al*., 1990 mod.)

- all solutions and reaction-tubes should be autoclaved two times → RNase free

RNA isolation and purification:

1. incubate 15 µl SDS (25 %) + 1200 µl phenol in a 2 ml E-cup at 65 °C for 5 min
2. suspend 2 ml cell pellet in cold AE-buffer (600 µl)
3. add the suspended cells to hot SDS/phenol solution (see above) and vortex
4. incubation of the suspended cells in SDS/phenol for 10 min at 65 °C (frequently vortex)
5. centrifuge 2 ml E-cup with suspended cells for 15 min at 4 °C and 7500 rpm
6. carefully transfer the clear supernatant in a new 2 ml E-cup and add 100 µl 2 M sodium acetate (pH 5.2) + 600 µl phenol (vortex)
7. see above step 5
8. see above step 6
9. see above step 5
10. carefully transfer the clear supernatant in a new 2 ml E-cup
11. add 2.5 volumes of ice cold ethanol (96 %) and vortex
12. at least 2 h incubation at -20 °C
13. sedimentation of RNA by centrifugation (13000 rpm, 4 °C, 1 h)
14. discard supernatant and wash the pellet with ice cold ethanol (70 %) (centrifugation: 13000 rpm, 4 °C, 5 min)
15. discard supernatant and dry pellet under laminar flow (approx. 30 min)
16. suspend the pellet in 15 µl TE buffer (pH 8)
17. storage the RNA at -70 °C

Dnase I treatment of RNA samples:

1. add to RNA sample (15 µl): 180 µl DNase I buffer (pH 7.5) + 5 µl Dnase I (10 U/µl)
2. incubation at 37 °C for 30 min
3. add 15 µl 2 M sodium acetate (pH 5.2) + 500 µl phenol
4. centrifugation for 15 min, 4 °C, 7500 rpm
5. carefully transfer the clear supernatant in a new 2 ml E-cup
6. add 2.5 volumes of ice cold ethanol (96 %) and vortex
7. at least 2 h incubation at -20 °C
8. sedimentation of RNA by centrifugation (13000 rpm, 4 °C, 1 h)
9. discard supernatant and wash the pellet with ice cold ethanol (70 %) (centrifugation: 13000 rpm, 4 °C, 5 min)
10. discard supernatant and dry pellet under laminar flow (approx. 30 min)
11. suspend the pellet in 15 µl TE buffer (pH 8)
12. storage the RNA at -70 °C

buffers and solutions:

AE buffer:	sodium acetate (waterfree)		164 mg
	EDTA		37 mg
	A. dest	ad	100 ml
	(adjust of pH at 5.5 with acetic acid)		
sodium acetate buffer:	sodium acetate (waterfree)		16,41 g
	A. dest	ad	100 ml
	(adjust of pH at 5.2 with acetic acid)		
TE (10/1) buffer:	1 M Tris HCl (pH 8)		1200 µl
	0,2 M EDTA (pH 8)		600 µl
	A. dest	ad	120 ml
Dnase I buffer:	Tris		480 mg
	$MgCl_2$		120 mg
	A. dest	ad	100 ml
	(adjust of pH at 5.5 with HCl)		