Deacylation and precipitation of tRNA

Deacylation of tRNA by treatment of total RNA

1. Set up reaction as follows:

Table 1: Representation of the composition of tRNA deacylation

Component	reaction
Deacylated RNA	total volume
3 M NaAC	1/10 volume of RNA total volume
EtOH	2 volume of RNA total volume

2. Rest for 40 min at 37°C

Ethanol precipitation of total RNA

1. Set up reaction as follows:

Table 2: Representation of the composition of ethanol precipitation of total RNA

Component	reaction
Deacylated RNA	total volume
3 M NaAC	1/10 volume of RNA total volume
EtOH	3 volume of RNA total volume

2. Rest at least 2 h at a temperature of -20°C
3. Centrifugation for 10 min at room temperature and full speed
4. Discard supernatant, dry pellet at room temperature for 10 minutes
5. Resuspend in 50 mM Tris-HCl (pH 8.0) and 2 mM $MgCl_2$
6. Adjust RNA concentration to 50 ng/µl