ExtractNow™ RNA Mini Kit

Protocol 1: RNA extraction from tissue/biopsy samples

C_W D_W
reconstitute with absolute ethanol

disrupt tissue

+ ≤ 20 mg tissue
+ 450 µl D_L

Proceed with the next step or store the sample in Lysis Buffer D at –20 °C.

max. speed / 1 min
proceed with complete supernatant

Protocol 2: RNA extraction from eukaryotic cells

C_W D_W
reconstitute with absolute ethanol
≤ 5 x 10^6 cells

+ 400 µl D_L
2min
resuspend 20 sec
3 min

Note:
ensure that all cells are completely lysed (no visible cell clumps) before you proceed to the next step.

Protocol 3: RNA extraction from bacteria

C_W D_W
reconstitute with absolute ethanol
≤ 5 x 10^9 bacteria

prepare stock solution of lysozyme

pellet cells for 2 - 5 min at 5000 × g
discard supernatant

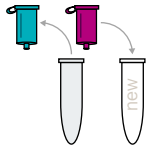
+ resuspend in 100 µl TE
+ 2 µl (20 mg/ml)
or
6 µl (50 mg/ml)
lysozym stock solution

mix
incubate until the solution becomes clear or viscous

+ 450 µl D_L
3 min

+ supernatant
10,000 × g for 2 min

Important:
The flow-through contains the RNA.
Thus do not discard the flow-through!



+ equal volume 70 % ethanol to the flow-through

mix

+ load flow-through/ ethanol mix
10,000 × g
+ for 1 min

change tube

+ 500 µl
10,000 × g
for 1 min
change tube
C_W

+ 700 µl
10,000 × g
for 1 min
change tube
D_W

10,000 × g
for 3 min
change tube

+ 30 to 80 µl
RNAse-free water
1 min

6000 × g for 1 min

store the extracted RNA at –20 °C or at –80 °C for long time storage.

+ add
vortex
incubate
centrifuge