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Total RNA Extraction Kit

Quick Start Guide

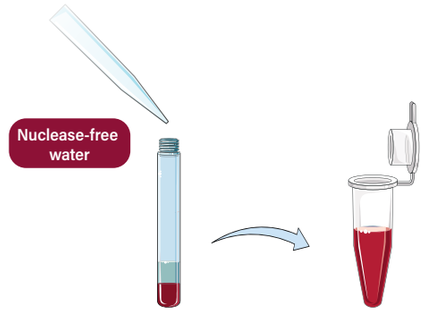
Part A RNA Extraction

1 Thaw Paxgene tube for 2 hours at room temperature.

Spin down PAXgene® tube at 5000g for 10 mins. Discard supernatant.

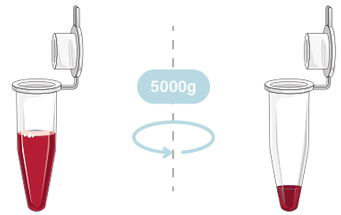
2 Resuspend pellet in 1.85ml nuclease-free, sterile water.

Transfer suspension to microfuge tube.

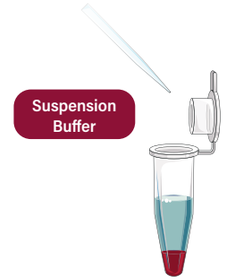


3 Spin down tube again at 5000g for 10 mins.

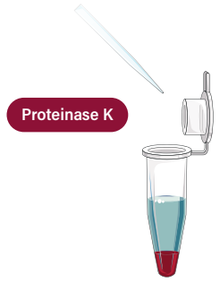
Discard supernatant.



4 Resuspend pellet in 300µl of Suspension Buffer.

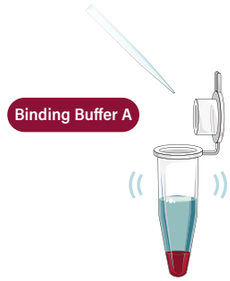


5 Add 40µl of Proteinase K.



6 **Add 300µl of Binding Buffer A** and mix by pipetting.

Vortex tube for 15 minutes with a vortex shaker at moderate speed.

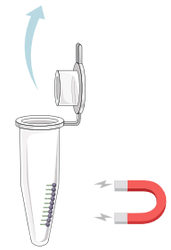


7 **Add 40µl of Binding Beads A** and mix by pipetting.

Vortex tube for 10 minutes with a vortex shaker.

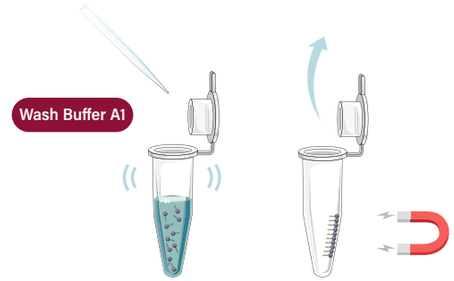
8 **Place tube on magnetic rack for 2 mins** to capture the RNA-bead complex.

Discard supernatant.



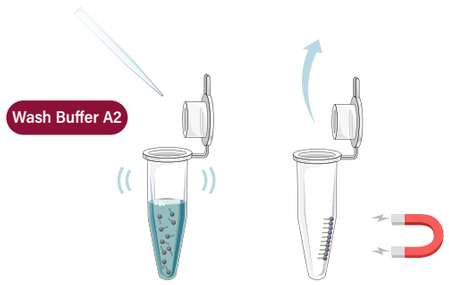
9 Resuspend beads in 600µl Wash Buffer A1. Mix by vortex shaker for 5 mins at moderate speed.

Place tube on magnetic rack for 2 mins to capture beads, then discard supernatant.



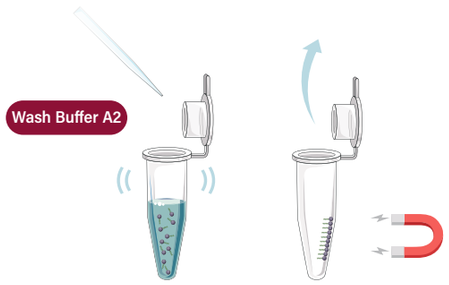
10 Resuspend beads in 600µl Wash Buffer A2. Mix by pipetting 20x.

Place tube on magnetic rack for 2 mins, then discard supernatant.



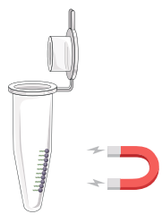
11 Repeat washing step with 600µl Wash Buffer A2.

Place tube on magnetic rack for 2 mins, then discard supernatant.



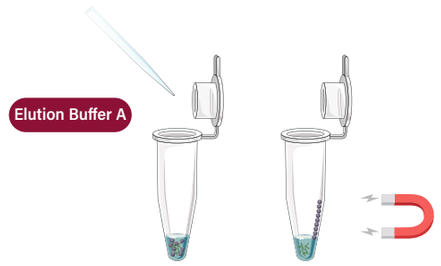
12 Dry beads on magnetic rack for 2 mins after the third wash.

Remove any wash buffer left at the bottom of tube at the end of drying step.



13 **Add 90µl of Elution Buffer A.**
Pipette 20x and incubate for 5 mins.

Place tube on magnetic rack for 5 mins.



14 Transfer supernatant to clean microfuge tube.

! For part B only 50µl of the elution is required.

Proceed to part B DNase Treatment

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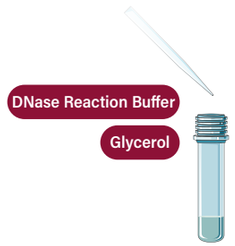
Part B DNase Treatment

15 Add 100µl of DNase Reaction Buffer to DNase pellet and then add 100µl of glycerol.

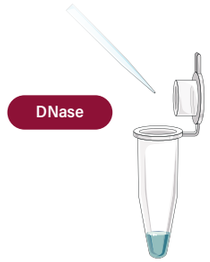
Mix by gently inverting the tube.

Reconstituted pellet must be stored at -20 °C.

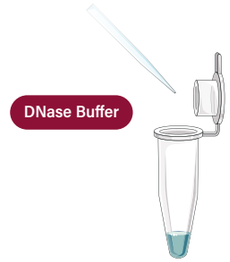


16 For each RNA sample, **prepare DNase buffer by adding 10µl of DNase prepared in the previous step to 40µl of DNase Reaction Buffer** in a microfuge tube.

Mix DNase with buffer by gently inverting the tube a few times.

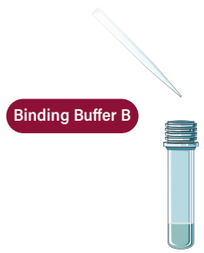


17 Add 50µl of DNase buffer to 50µl of RNA sample in a microfuge tube.



18 Gently shake microfuge tube and incubate at room temperature for 25 minutes.

19 Mix 40µl of Binding Beads B with 400µl Binding Buffer B.

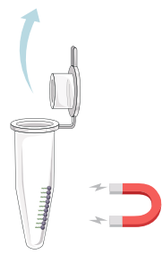


20 Add 400µl of Binding Bead and Binding Buffer mixture to microfuge tube and mix by pipetting.

21 Vortex for 5 mins in a vortex shaker.

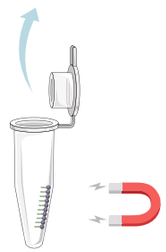
22 Place tube on magnetic rack for 2 mins.

Discard supernatant.



23 Resuspend beads in 600µl Wash Buffer B1. Mix by pipetting 20x.

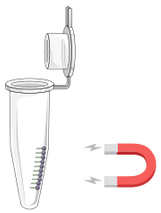
24 Place tube on magnetic rack, wait for 2 minutes and discard supernatant.



25 Repeat washing step with Wash Buffer B1.

26 Dry beads on magnetic rack for 2 mins after the second wash.

Remove any wash buffer left at the bottom of tube at the end of drying step.



27 Add 50µl of Elution Buffer B to tube.

Mix by pipetting 20x and incubate for 5 mins.

28 Place tube on magnetic rack, wait for 5 mins and then transfer supernatant to clean tube.