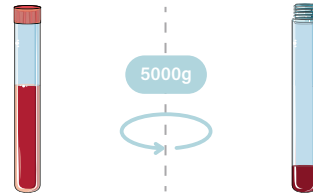
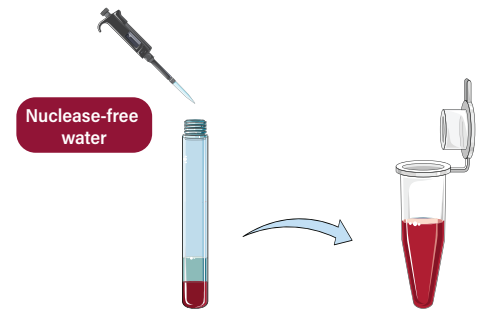


1 Spin down PAXgene® tube at 5000g for 10 mins. Discard supernatant.

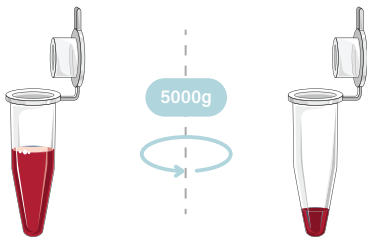


2 Resuspend in 1.8ml of nuclease-free, sterile water.

Transfer suspension to nuclease-free 2ml tube.

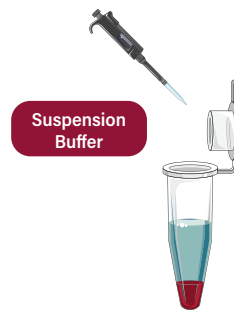


3 Spin down tube at 5000g for 10 mins. Discard supernatant.



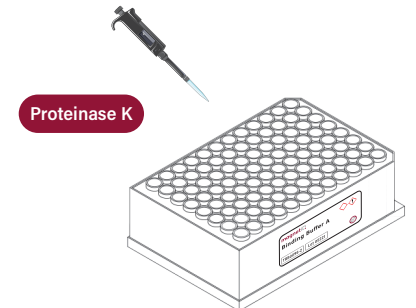
4 Resuspend pellet in 300µl of Suspension Buffer by pipetting or vortexing.

Make sure pellet is fully resuspended in the buffer.

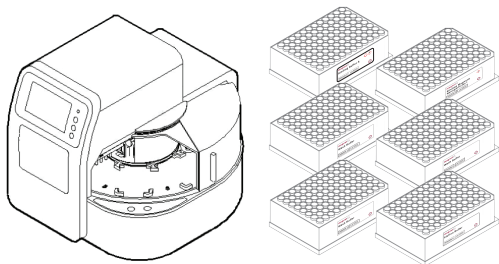


5 To the Binding Buffer A plate, add 40µl Proteinase K to each sample well.

Transfer the 300µl resuspension into a well on the Binding Buffer A plate. Dispense directly into the Binding Buffer, not on the walls.



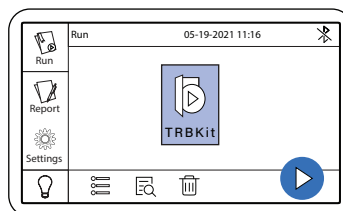
6 Place Binding Buffer A plate into purification system with the Binding Magnetic Nanoparticles A plate, three (3) Wash Buffer plates and one Elution Buffer plate.



! Place tip combs on Binding plate or in dedicated tip plate, according to machine's specifications.

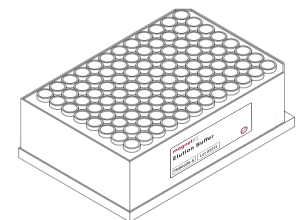
7 Run the Total RNA PAXgene protocol.

15 min	proteinase digestion
5 min	binding
5 min	wash #1
3 min	wash #2
3 min	wash #3
3 min	drying
5 min	elution



8 When the protocol completes, remove the Elution Buffer plate for use in part B: DNase treatment.

Discard the tip comb and other plates from the purification system.

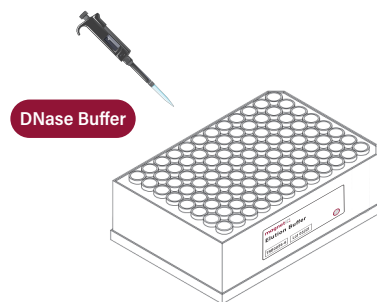


9 Prepare DNase buffer by adding one volume of DNase to 4 volumes of DNase Buffer. Mix DNase with buffer by gently inverting the tube a few times.

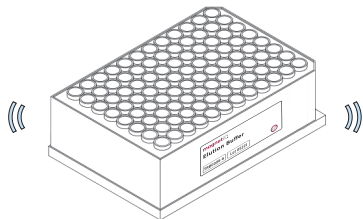
Make sure DNase is perfectly mixed by observing disappearance of smears within reaction buffer when inverting the tube.



10 To the elution plate from part **A** add 50µl of DNase Buffer. Dispense directly into the elution buffer not on the walls. If there are any droplets of elution buffer left on the walls, slide them to the well bottom by gently shaking or tapping the plate on a solid surface.



11 Gently shake the elution plate for 10 seconds by hand and incubate at room temperature for 20 min.

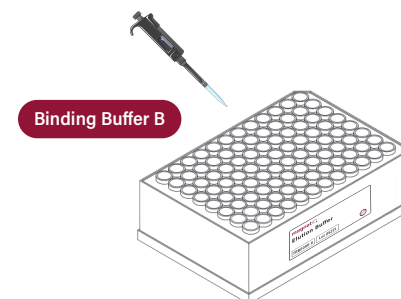


12 Mix 4ml of Binding Magnetic Nanoparticles **B** solution to 40ml of Binding Buffer B.

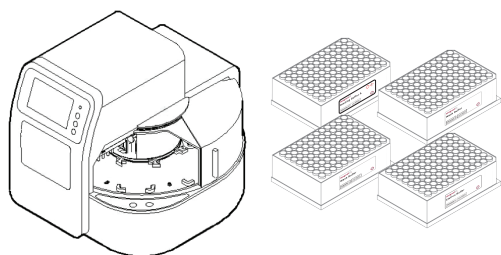


! This is for 96 preps.

13 Add 400µl of Binding Buffer B to each well of the Elution Buffer **A** plate.



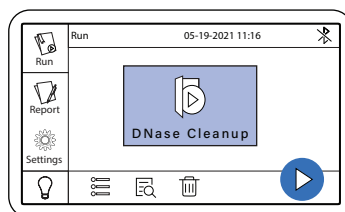
14 Place the Elution plate from part A into purification system purification system with two (2) Wash Buffer plates and one Elution Buffer **B** plate.



! Place tip combs on Elution plate or in dedicated tip plate, according to machine's specifications.

15 Run the Total RNA DNase CleanUp Protocol.

5 min	binding
1 min	wash #1
1 min	wash #2
2 min	drying
2 min	elution



16 When the protocol complete, remove the final Elution Buffer **B** plate containing the purified RNA.

Discard the tip comb and remaining plates from the purification system.

