NucleoSpin® RNA – Support Protocol – RNA from PAXgene® tubes

Protocol Details

Application	RNA isolation from PAXgene® tubes
Kit	NucleoSpin® RNA
REF	740955 (.10/ .50/ .250)
Protocol name	Support Protocol – RNA from PAXgene® tubes Rev01

Protocol Steps

Steps	Procedure
1. Harvest sample	Centrifuge the PAXgene® Blood RNA Tube for 10 min at 3000 – 5000 x g using a swing-out rotor.
2. Wash pellet	Remove the supernatant by decanting or pipetting. Add **4 mL RNase-free water** to the pellet, and close the tube using a fresh secondary Hemogard closure. Thoroughly resuspend the pellet by vortexing, and centrifuge for 10 min at 3000 – 5000 x g. Remove and discard the entire supernatant. Gently decant the supernatant, and blot on a paper towel. Thoroughly resuspend the pellet in **300 µL RNase-free water** by pipetting. Do not vortex.
3. Lyse sample	Pipette the sample into a 1.5 ml microcentrifuge tube. Add **300 µL RA1** supplemented with 3 µL ß-Mecaptoethanol, and **40 µL Proteinase K**. Mix by vortexing, and incubate for 20 min at 55 °C using a shaker incubator, heating block, or water bath.
4. Filtrate sample	Clear the lysate by filtration through NucleoSpin® Filter (violet rings). Place the NucleoSpin Filter in a Collection Tube (2ml), apply the mixture and centrifuge for 1 min at 11,000 x g.
5. Adjust RNA binding conditions	Discard the NucleoSpin® Filter and transfer flow-through (cleared lysate) to a new reaction tube. Add **300 µL ethanol** (96 %) to the homogenized lysate and mix by pipetting up and down (5 – 10 times).
6. Transfer lysates	Load the lysate to the NucleoSpin® RNA column (light blue ring). Proceed with step 5 of the standard protocol (‘Bind RNA’).

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Reagents, consumables, and equipment to be supplied by user

Equipment	Content	REF
Reagents		
Proteinase K	2x50 mg Proteinase K 8 ml Proteinase Buffer PB	740506
Equipment		
Water bath or heating block		

Trademarks

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