RNA Enrichment Protocol

Reagents and Supplies

Tips
Pipettes set
Eppendorf tubes 1.5mL
Tube 0.2mL
Thermocycler
Thermoblock
Centrifuge
Zymo-Spin IC - RNA Clean & Concentrator kit
Filter 0.22µm
Turbo DNAse
Viral RNA Extraction Kit

Swab and Serum samples preparation

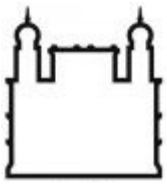
-Centrifuge the sample at 16000g for 2min. Remove the overnatant and filter it in a 0.22µm filter.
-Proceed with the viral RNA extraction protocol
-WARNING: Do not use RNA Carrier.
-Use 60uL of water for elution;

Tissues samples preparation

-The tissue will be macerated in lysis buffer from the extraction kit, and then centrifuged at 16000g for 2min. Remove the supernatant and filter it in a 0.22µm filter.
-Proceed with the viral RNA extraction protocol
- WARNING: Do not use RNA Carrier.
-Use 60µL of water for elution;

DNAse I treatment

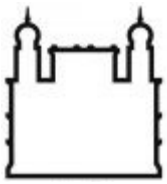
- In a new 1.5mL eppendorf tube add 44µL of extracted RNA, 5µL of 10X TURBO DNase Buffer and 1µL of TURBO DNase;
-Incubate the tube at 37ºC for 30 minutes;
-After incubation, add to the 100µl sample of RNA Binding Buffer. Use a vortex to mix for 5 seconds and centrifuge with a nanospin centrifuge;
-Then add 150µL of 100% ethanol. Use a vortex to mix for 15 seconds and centrifuge with a nanospin centrifuge;
-Transfer 300µL of the solution to a new Zymo-Spin IC column tube and centrifuge at 16000g for 30 seconds. Discard the filtrate.
-Add 400µl of RNA Prep Buffer and centrifuge to 16000g for 30 seconds. Discard the filtrate.



-Add 700µl RNA Wash Buffer and centrifuge to 16000g for 2 minutes.
-Discard the filtrate and transfer the Zymo-Spin IC column to a new 1.5mL eppendorf tube;
-Add ~11µl DNase/RNase Free Water and incubate at room temperature for 1 minute.
-Centrifuge at 16000g for 30 seconds and store the RNA tube on ice until next steps or store at -70ºC.

References

Chiu C, Greninger AL, Naccache SN, Federman S, Yu G, Mbala P, Bres V, Stryke D, Bouquet J, Somasekar S, Linnen JM. Rapid metagenomic identification of viral pathogens in clinical samples by real-time nanopore sequencing analysis.



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