Laboratory Operating Procedure

Extraction of total RNA from concentrated viral solution

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LOP-Version: 1

Attachments:

Introduction

The here described method is used for extraction of total nucleic acid (NA) from liquid samples. This protocol is a combination of bioMerieux NucliSENS kit and Zymo research kit. The lysis buffer broke viral capsids under chaotropic salt conditions, and then NA are captured using magnetic silica beads facilitating capturing the nucleic acid and washes.
The second kit using a DNAse treatment remove DNA, purify and concentrate RNA.

Sample Material

Liquids, as for example last step of PEG method (Lop).

Equipment and Reagents

Equipment

- Refrigerated centrifuge (for 1.5 ml and 2 ml tubes)
- Vortexer
- Pipettes
- Disposable gloves
- 1.5 ml and/or 2 ml tubes
- Sterile, RNase-free pipet tips (with aerosol barriers to prevent cross contamination)
- nucliSENS® miniMAG™(bioMerieux ref.200 305): instrument for the magneticextraction of nucleic acids. In the absence of NucliSENS miniMAG, extractions can be performed with magnetic stand
- (magnetic stand)
- Thermoshaker

Reagents

- NucliSens magnetic extraction reagents (bioMerieux; cat . No 200 293)
- Nuclisens lysis buffer (bioMerieux; cat. No 280134)
- RNA Clean & Concentrator -5 (Zymo Research; No. R1015)
- Turbo DNAse (life technology; cat. No. 1907M)

General remarks

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Literature

- RNA Clean & Concentrator – 5 Instruction Manual (Zymo research : http://www.zymoresearch.com/downloads/dl/file/id/141/r1013i.pdf)
- Turbo DNase (ambion, Life Technology, https://tools.thermofisher.com/content/sfs/manuals/cms_055740.pdf)

Before getting started

- Equilibrate samples and reagents at room temperature (RT)

Procedure

1.
- Add 10 mL of NucliSENS lysis buffer directly in the tube containing volume of liquid (no more than 1 mL)

2.
- Incubate for 10 min at RT
- Mix silica solution using a vortexer
- Add 140 µL of silica and mix briefly
- Incubate for 10 min at RT

3.
- Place the tube one magnetic stand to separate the silica from the supernatant.
- Discard supernatant
- Add 400 µL of washing buffer 1 and mix by pipetting
- Transfer 400 µL solution in a clean tube one NucliSENS Mini Mag
- Wash 30 sec (Step1) and discard supernatant (with magnet)
- Add 400 µL of washing buffer 1 and mix by pipetting (without magnet)
- Wash 30 sec (Step1) and discard supernatant (with magnet)
- Add 500 µL of washing buffer 2 and mix by pipetting (without magnet)
- Wash 30 sec (Step1) and discard supernatant (with magnet)
- Add 500 µL of washing buffer 2 and mix by pipetting (without magnet)
- Wash 30 sec (Step1) and discard supernatant (with magnet)
- Add 500 µL of washing buffer 3 and mix by pipetting (without magnet)
- Wash 30 sec (Step1) and discard supernatant (with magnet)
- Add 100µL of elution buffer
- Shake 10 min, at 72°C, at 1400 rpm in thermoshaker

4.
- Place the tube one magnetic stand and collect 100µL nucleic acid extract (extract can be stoke at -20°C)

5.
- Add 12,5 µL 10X Turbo DNase Buffer and 12,5 µL Turbo DNase (2Units/µL)
- Incubate 30min at 37°C
- Add 200µL of RNA Binding Buffer and mix.
- Add an 300µL of ethanol (95-100%) and mix
- Transfer the sample to the Zymo-Spin™ IC Column in a collection tube
- Centrifuge for 30 seconds, discard the flow-through.
- Add 400 µl of RNA Prep Buffer to the column
- Centrifuge for 30 seconds. Discard the flow-through.
- Add 700 µl of RNA Wash Buffer to the column
- Centrifuge for 30 seconds. Discard the flow-through.
- Add 30 µl DNase/RNase-Free Water directly to the column matrix and centrifuge for 30 seconds.

The eluted RNA can be used immediately or stored at -70°C.