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Total RNA Purification Kit - Supplementary Protocol

Product # 17200

Total RNA Isolation from Cerebrospinal Fluid (CSF) using Total RNA Purification Kit (#17200)

Component Required

Component	Product #
Total RNA Purification Kit	17200

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96 – 100 % Ethanol
- *Optional*: MS2 RNA (0.8 µg/µl). (Roche, Cat. No. 10165948001)

Notes Prior to Use

- Bodily fluids of all human and animal subjects is considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with cerebrospinal fluid (CSF).
- It is recommended that no more than 200 µL of CSF be used in order to prevent clogging of the column.
- Avoid multiple freeze-thaw cycle of the CSF sample. Aliquot to the appropriate volume for usage prior to freezing.
- The yield of RNA from CSF is very low and is highly variable. In general, the expected yield could vary $\leq$ 1 ng per 100 µL CSF used. Regular spectrophotometry quantification may not be applicable for such low amount of RNA. Nonetheless, these isolated RNA could still be used effectively in different downstream applications such as Whole transcriptome amplification, RT-qPCR or microarrays.
- It is important to work quickly during this procedure.

1. Cell Lysate Preparation from Cerebrospinal Fluid (CSF)

a. Transfer up to 200 µL of CSF to an RNase-free microcentrifuge tube (not provided).
b. Add 300 µL of Lysis Solution to every 100 µL of CSF. Mix by vortexing for 10 seconds.
c. **Optional:** Add 0.7 µL of 0.8 µg/µl MS2 RNA per sample.

Note: The use of MS2 RNA could increase the consistency of downstream applications such as RT-PCR. However, the use of MS2 RNA is not recommended for applications involving global gene expression analysis such as microarrays or sequencing.

d. Add 400 µL of 96 – 100% ethanol (provided by the user) to every 400 µL of the lysate (equivalent to every 100 µL CSF used). Mix by vortexing for 10 seconds. Proceed to Step 2 below.

2. Binding RNA to Column

a. Assemble a column with one of the provided collection tubes
b. Apply up to 600 µL of the lysate with the ethanol (from Step 1) onto the column and centrifuge for 1 minute.

Note: Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Depending on your lysate volume, repeat Step 2b and 2c as necessary.

Optional Step:

Norgen's Total RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional On-Column DNA Removal Protocol is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol.

3. Column Wash

a. Apply 400 µL of 96 – 100% ethanol (provided by the user) to the column and centrifuge for 1 minute.

Note: Ensure the entire ethanol solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

b. Discard the flowthrough and reassemble the spin column with its collection tube.
c. Repeat steps 3a and 3b to wash column a second time.
d. Wash column a third time by adding another 400 µL of 96 – 100% ethanol (provided by the user) and centrifuging for 1 minute.
e. Discard the flowthrough and reassemble the spin column with its collection tube.
f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

4. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 30 - 50 µL of Elution Solution to the column.
c. Centrifuge for 2 minutes at 200 x g (~2,000 RPM), followed by 1 minute at 14,000 x g (~14,000 RPM).
d. Pipet the eluted RNA back to the top of the column and repeat Steps **4b** and **4c** one more time.

Note: The repeat elution will increase the recovery of the RNA

5. Storage of RNA

The purified RNA sample may be stored at –20°C for a few days. It is recommended that samples be placed at –70°C for long term storage.

Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website (www.norgenbiotek.com) or through email at techsupport@norgenbiotek.com.

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