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Plasma/Serum RNA Purification Mini Kit - Supplementary Protocol

Product # 55000

miRNA Isolation from ExoComplete™ 96-Well Plate Kit Working Lysis Buffer (Hitachi) using Plasma/Serum RNA Purification Mini Kit (#55000)

Component Required

Component	Product #
Plasma/Serum RNA Purification Mini Kit	55000

Customer-Supplied Reagents and Equipment

- Benchtop microcentrifuge
- 96 – 100 % Ethanol
- Vortexer
- Micropipettors
- Optional: β - Mercaptoethanol

Storage Conditions and Product Stability

All buffers should be kept tightly sealed and stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. It is recommended to warm Lysis Buffer A for 20 minutes at 60°C if any salt precipitation is observed.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. **Lysis Buffer A** contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions. Plasma or serum 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 plasma or serum.

Procedure

Notes Prior to Use:

- All centrifugation steps are performed at room temperature.
- Ensure that centrifuge tubes used are capable of withstanding the centrifugal forces required.
- The provided spin columns are optimized to be used with a benchtop centrifuges and not to be used on a vacuum apparatus
- Most standard benchtop microcentrifuges will accommodate Norgen's Micro Spin Columns.
- Centrifuging Norgen's Spin Columns at a speed higher than recommended may affect RNA yield.
- Centrifuging Norgen's Spin Columns at a speed lower than recommended will not affect RNA yield. However, centrifugation at a lower speed may require longer time for the solutions to pass through the spin column
- Ensure that all solutions are at room temperature prior to use.
- It is highly recommended to warm up **Lysis Buffer A** at 60°C for 20 minutes and mix well until the solutions become clear again if precipitates are present.
- Prepare a working concentration of the **Wash Solution A** by adding 42 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated Wash Solution A. This will give a final volume of 60 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- The use of β-mercaptoethanol in lysis is highly recommended to isolate RNA for sensitive downstream applications. Add 10 µL of β-mercaptoethanol (provided by the user) to each 1 mL of **Lysis Buffer A**.
- Ensure that samples have not undergone more than one freeze-thaw cycle, as this may lead to RNA degradation.
- It is recommended to not work with samples that were hemolyzed as this will affect the RNA profile outcome
- ***This kit is suitable for the isolation of RNA from serum or plasma prepared from blood collected on either EDTA or citrate. Plasma samples prepared from blood collected on heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR.***
- ***If any of the solutions do not go through the Spin Columns within the specified centrifugation time, spin for an additional 1-2 minutes until the solution completely passes through the column. Do NOT exceed the centrifugation speed as this may affect DNA yield.***

1. Follow the procedure outlined in the **ExoComplete™ 96-Well Plate Kit** instruction manual for the **EMV Isolation and mRNA Hybridization** followed by **cDNA synthesis** up to **Step 4**.

2. Collect and transfer the 60 µL lysate into a 2 mL tube (provided by the user) and add 140 µL Nuclease-free water (provided by the user).

Note: If the lysate volume was not 60 µL, simply bring the volume of the lysate up to 200 µL using Nuclease-free water and proceed.

3. To the lysate from **Step 2** add 600 µL of **Lysis Buffer A**. Mix well by vortexing for 10 seconds.

4. Add 800 µL of 96-100% ethanol (provided by the user). Mix well by vortexing for 10 seconds.

5. Transfer 650 µL of the mixture from **Step 4** into a Micro Spin column. Centrifuge for **1 minute at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.

6. Repeat **Step 5** two more times until all the mixture from **Step 4** has been transferred to the Micro Spin column.

7. Apply 600 µL of **Wash Solution A** to the column and centrifuge for **30 seconds at 3,300 *x g* (~6,000 RPM)**. Discard the flowthrough and reassemble the spin column with its collection tube.

8. Repeat **step 7** one more time, for a total of two washes.

9. Spin the column, empty, for **2 minutes at 13,000 *x g* (~14,000 RPM)**. Discard the collection tube.

10. Transfer the spin column to a fresh 1.7 mL Elution tube. Apply from 10 µL up to 25 µL of **Elution Solution A** to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

11. For maximum recovery, transfer the eluted buffer back to the column and let stand at room temperature for 2 minutes. Centrifuge for **1 minute at 400 x *g* (~2,000 RPM),** followed by **2 minutes at 5,800 x g (~8,000 RPM)**.

    - ***Plasma/Serum Exosomal miRNA is ready for the downstream application of your choice. For an explanation of expected yields and recommendations for quantification of the RNA, please refer to Appendix B of the full version manual available on our website***

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 or through email at techsupport@norgenbiotek.com.