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RNA Clean-Up and Concentration Micro-Elute Kit - Supplementary Protocol

Product # 61000

NGS Library Clean-Up and Concentration (removal of adapters, salts and enzymes)

Notes Prior to Use

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the Wash Solution A by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle(s) containing the concentrated Wash Solution A. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- It is important to work quickly during this procedure.

1. Sample Preparation

a. Adjust the volume of the sample to 100 µL by adding RNase-free water.
b. Add 300 µL of **Buffer RL** to the sample. Mix by vortexing
c. Add 50 µL of 96 – 100% ethanol (provided by the user) to the mixture from **Step 1b.** Mix by vortexing for 10 seconds.

2. Column Activation and Sample Binding to Column

a. Assemble a column with one of the provided collection tubes.
b. Apply 500 µL of **Column Activation Solution** onto the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).
c. Discard the flowthrough. Reassemble the spin column with its collection tube.
d. Apply the sample with the ethanol (from **Step 1c**) onto the **activated column** and centrifuge for 1 minute at ≥3,500 x g (6,000 RPM).
e. Discard the flowthrough. Reassemble the spin column with its collection tube.

3. Column Wash

a. Apply 600 µL of **Wash Solution A** to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM).

Note: Ensure the entire Wash Solution A 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 the column a second time.
d. Discard the flowthrough and the collection tube and reassemble the spin column with a new collection tube.
e. Spin the column for 2 minutes at 14,000 x g (~14,000 RPM) in order to thoroughly dry the resin. Discard the collection tube.

4. DNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.

b. Add 8 – 15 µL of **Elution Solution A** to the column.

Note: For maximum concentrations, the 8 µL elution volume may be used. For maximum recovery, the 15 µL elution volume is recommended

c. Centrifuge for 1 minute at **200 x *g* (~2,000 RPM)**, followed by 2 minutes at **14,000 x *g* (~14,000 RPM)** Note the volume eluted from the column. If the entire volume has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

Note: For maximum recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 4b and 4c**).

5. Storage of DNA

The purified sample may be stored at –20°C.

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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