Cells and Tissue DNA Isolation Micro Kit - Supplementary Protocol

DNA Purification from Blood Preserved on a Neoteryx Mitra® Microsampling Device

Component	Neoteryx Part Number
Cells and Tissue DNA Isolation Micro Kit	114 (Evaluation Kit 118)

Customer-Supplied Reagents and Equipment

- Neoteryx Mitra 10 µL Microsampling Device
- Benchtop microcentrifuge
- 96 – 100 % Ethanol
- Phosphate buffered saline (PBS)
- 56°C water bath or incubator

Notes Prior to Use

- Bodily fluids of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with bodily fluids such as blood.
- Please refer to the Mitra device manual for instructions for blood collection

1. Cell Lysate Preparation from Neoteryx Mitra Microsampling Device

a. Dispense one or two tips of the Neoteryx Mitra™ Microsampling device into a 1.5 mL microfuge tube.
b. Add 200 µL of phosphate buffered saline (PBS) and vortex for 10 seconds.
c. Add 20 µL of Proteinase K and 300 µL of **Lysis Buffer B** and vortex for 10 seconds.
d. Incubate at 56° for 20 minutes.
e. Transfer the lysate into a clean microfuge tube.
f. Add 250 µL of 96-100% ethanol and vortex to mix.

2. Binding to Column

a. Assemble a Micro Spin Column with a provided collection tube. Apply the mixture to the spin column assembly. Cap the column, and centrifuge the unit for 2 minutes at 6,000 x g (~ 8,000 RPM).
b. **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 at 14,000 x g (~14,000 RPM) for 2 minutes.
c. After centrifugation, discard the flowthrough, and reassemble the spin column with its collection tube.

3. Washing Bound DNA

a. Apply 500 µL of Solution WN (ensure ethanol was added) to the column and centrifuge for 1 minute at 6,000 x g (~8,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
b. **Note:** Ensure the entire wash 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.

c. Apply 500 µL of Wash Solution A (ensure ethanol was added) to the column and centrifuge for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
d. Wash column another time by adding 500 µL of Wash Solution A and centrifuging for 1 minute at 14,000 x g (~14,000 RPM). Discard the flowthrough and reassemble the spin column with its collection tube.
e. Spin the column for 2 minutes in order to thoroughly dry the column at 14,000 x g (~14,000 RPM). Discard the collection tube.

4. Elution of Clean DNA

a. Place the column into a fresh 1.7 mL microcentrifuge tube (provided by the user).
b. Add 30 µL of Elution Buffer B to the column.
c. Incubate at room temperature for 1 minute.
d. Centrifuge for 1 minute at 6,000 x g (~8,000 RPM) followed by 1 minute at 14,000 x g (~14,000 RPM).

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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

Total RNA Purification Plus Micro Kit - Supplementary Protocol

Total RNA Isolation from Neoteryx Mitra® Microsampling Device

Component	Neoteryx Part Number
Total RNA Purification Plus Micro Kit	113 (Evaluation Kit 117)

Customer-Supplied Reagents and Equipment

- Neoteryx 10 µL Mitra Microsampling Device
- Benchtop microcentrifuge
- 96 – 100 % Ethanol
- Water bath or heat block set at 42°C

Notes Prior to Use

- Bodily fluids of all human and animal subjects are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with bodily fluids such as blood.
- Please refer to the Mitra device manual for instructions for blood collection
- It is important to work quickly during this procedure.

1. Cell Lysate Preparation from Neoteryx Mitra Microsampling Device

a. Dispense one or two tips of the Neoteryx Mitra Microsampling device into a 1.5 mL microfuge tube
b. Add 300 µL of **Buffer RL** to the tube containing the tip(s). Mix by vortexing for 15 seconds.
c. Incubate the sample at 42°C for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
d. At the end of the incubation, vortex the tube one more time for 15 seconds. Centrifuge the tube at 14,000 RPM (~14,000 x *g*) for 1 minute. Transfer the supernatant to a fresh 1.5 mL microfuge tube.
e. **Proceed to Step 2.**

2. Genomic DNA Removal

- This kit is provided with 2 separate columns. When columns are removed from the labelled bags they are supplied in they can easily be identified as follows:
  - gDNA Removal Columns - column has blue and white contents
  - RNA Purification Micro Columns – column has grey and white contents

- Assemble a gDNA Removal Column with one of the provided collection tubes.
- Apply the entire lysate prepared from Section 1 onto the column and centrifuge at 14,000 x g (~14,000 RPM) 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. **Retain the flowthrough for RNA Purification (Section 3)**. The flowthough contains the RNA and should be stored on ice or at -20°C until the RNA Purification protocol is carried out.
d. Dispose of the gDNA Removal Column with the bound gDNA.

3. Binding RNA to Column

a. To every 100 µL of flowthrough from Step **2c**, add 60 µL of 96 – 100 % Ethanol. Mix by vortexing.

Note: For example, for 300 µL of flowthrough, add 180 µL of 96 – 100 % Ethanol

b. Assemble an RNA Purification Micro Column with one of the provided collection tubes.
c. Apply up to 600 µL of the lysate with the ethanol onto the column and centrifuge at 3,500 x g (~6,000 RPM) 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.

d. Depending on your lysate volume, repeat Step **3b** and **3c** as necessary.

4. Column Wash

a. Apply 400 µL of **Wash Solution** to the column and centrifuge centrifuge at 14,000 x g (~14,000 RPM) for 1 minute.

Note: Ensure the entire wash 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 **4a** and **4b** to wash column a second time.
d. Repeat steps **4a** and **4b** to wash column a third time.
e. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

5. RNA Elution

a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
b. Add 40 µL of **Elution Solution** to the column.

Note: For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 20 µL is recommended

c. Centrifuge for 2 minutes at **200 x *g* (~2,000 RPM)**, followed by 1 minute 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 RNA recovery, it is recommended that a second elution be performed into a separate microcentrifuge tube (Repeat **Steps 5b and 5c**).

6. 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.

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6
Phone: (905) 227-8848
Fax: (905) 227-1061
Toll Free in North America: 1-866-667-4362

