Total RNA Purification from Plasma or Serum (ISOLATE II Biofluids RNA Kit)

Bioline

Abstract

Protocol for RNA Purification form Plasma or Serum, using the ISOLATE II Biofluids RNA Kit. This protocol includes the lysate preparation procedure.

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Guidelines

Before you start:

- 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.
- We recommend the use of this kit to isolate RNA from plasma or serum prepared by a standard protocol from non-coagulated, fresh whole blood using EDTA or sodium citrate as the anti-coagulant.
- Plasma prepared from fresh blood using heparin as an anti-coagulant is not suitable for use with this protocol.
- Due to the relatively low DNA content in plasma, the Genomic DNA Removal Column is not necessary for this protocol.
- It is recommended that no more than 200 µL of plasma or serum is used in order to prevent clogging of the column.

- Yields of RNA from plasma and serum is highly variable. In general, the expected yield

  could vary from 1 to 100 ng per 100 µL plasma or serum used. In addition, the expected A_{260}/A_{280} ratio as well as the A_{260}/A_{230} ratio will be lower (<1.8) than the normal acceptable range from other cells or tissues. Nonetheless, these isolated RNA can be effectively used in different downstream applications such as real-time PCR or microarrays.

- Avoid multiple freeze-thaw cycles of the plasma or serum sample. Aliquot out the appropriate volume for usage prior to freezing.

- It is important to work quickly during this procedure.

Please review the Guidelines under Genomic DNA removal and total RNA purification from all types of lysate for other important details.

Before start

Materials

ISOLATE II Biofluids RNA Kit BIO-52086 by Bioline

Protocol

Lysate Preparation from Plasma/Serum

Step 1.

Transfer up to 200 µL of plasma or serum to a 1.5mL RNase-free microcentrifuge tube (not supplied).

Lysate Preparation from Plasma/Serum

Step 2.

Add 300 µL of Lysis Buffer RX to every 100 µL of plasma or serum.

Lysate Preparation from Plasma/Serum

Step 3.

Mix by vortexing for 10s.

DURATION

00:00:10

Lysate Preparation from Plasma/Serum

Step 4.

Optional: Add 0.7µL of 0.8µg/µL MS2 RNA per sample.

NOTES

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

Lysate Preparation from Plasma/Serum

Step 5.

Add 400 µL of 96-100% ethanol to every 400 µL of lysate (equivalent to every 100µL plasma or serum used).

Lysate Preparation from Plasma/Serum

Step 6.

Mix by vortexing for 10s.

DURATION

00:00:10

Binding RNA to Column

Step 7.

Assemble an ISOLATE II RNA Column (black ring) with a provided Collection Tube.

Binding RNA to Column

Step 8.

Apply up to 600µL of the ethanolic lysate onto the column and centrifuge for 1 min at ≥ 3,500 x g.

DURATION

00:01:00

NOTES

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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 through, spin for an additional minute at 14,000 x g.

Binding RNA to Column

Step 9.

Discard the flow-through. Reassemble the spin column with its Collection Tube.

Binding RNA to Column

Step 10.

Depending on the lysate volume,repeat steps 8 and 9 as required.

RNA Column Wash

Step 11.

Apply 400 µL of 96-100% ethanol to the column and centrifuge for 1 min at 14,000 x g. (wash 1/3)

DURATION

00:01:00

NOTES

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Note: Ensure the entire wash buffer volume has passed through into the Collection Tube by inspecting the column. If the entire wash buffer volume has not passed through, spin for an additional minute at 14,000 x g.

RNA Column Wash

Step 12.

Discard the flow-through and reassemble the spin column with its Collection Tube. (wash 1/3)

RNA Column Wash

Step 13.

Apply 400 µL of 96-100% ethanol to the column and centrifuge for 1 min at 14,000 x g. (wash 2/3)

DURATION

00:01:00

NOTES

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Note: Ensure the entire wash buffer volume has passed through into the Collection Tube by inspecting the column. If the entire wash buffer volume has not passed through, spin for an additional minute at 14,000 x g.

RNA Column Wash

Step 14.

Discard the flow-through and reassemble the spin column with its Collection Tube. (wash 2/3)

RNA Column Wash

Step 15.

Wash column a third time by adding 400µL of 96-100% ethanol and centrifuge for 1 min

at 14,000 x g. (wash 3/3)

DURATION

00:01:00

RNA Column Wash

Step 16.

Discard the flow-through and reassemble the spin column with its Collection Tube. (wash 3/3)

RNA Column Wash

Step 17.

Spin the column for 2 min at 14,000 x g in order to dry the column thoroughly. Discard the

Collection Tube.

DURATION

00:02:00

RNA Elution

Step 18.
Place the column into a fresh 1.7mL Elution Tube.

RNA Elution

Step 19.
Add 50µL of RNA Elution Buffer to the column.

RNA Elution

Step 20.
Centrifuge for 2 min at 200 x g.

DURATION
00:02:00

RNA Elution

Step 21.

Centrifuge for 1 min at 14,000 x g. Note the volume eluted from the column. If the entire 50 µL has not been eluted, spin the column at 14,000 x g for an additional minute to elute the RNA.

DURATION
00:01:00

NOTES
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Note: For maximum RNA recovery, it is recommended to apply a second volume of RNA Elution Buffer and elute into the same microcentrifuge tube (repeat steps 19-21). Alternatively, re-apply the first eluate onto the column and re-elute into the same microcentrifuge tube (for high concentration).

Storage of RNA

Step 22.

The isolated RNA can be stored at -20°C for a few days or at -80°C (recommended) for long-term storage.

Warnings

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.

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Lysis Buffer RX contains guanidinium thiocyanate. This chemical is harmful in liquid form when in

contact with skin or ingested. If the solution is allowed to dry, the powder is harmful if inhaled.

CAUTION: Do not add bleach directly to solutions or sample preparation waste containing guanidinium salts. Reactive compounds and toxic gases can form. In the case of spillage, clean the affected area with a suitable laboratory detergent and water.

For detailed information, please consult the material data safety sheet (MSDS) available on our website at www.bioline.com.

Biofluids derived from all human and animal sources are considered potentially infectious. All necessary precautions recommended by the appropriate authorities in the country of use should be taken when working with biofluids.