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# Total RNA extraction from frozen placenta tissue

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Scott Lindsay-Hewett[1]

[1]University of California, San Diego

Human BioMolecular Atlas Program (HuBMAP) Method Development Community
Tech. support email: **Jeff.spraggins@vanderbilt.edu**

Scott Lindsay-Hewett
University of California San Diego





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**We use this protocol and it's working**

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## Abstract

This protocol describes the isolation of high-quality total RNA from frozen placenta tissue. Tissue is disrupted using a bead beater, and total RNA is isolated using the *mir*Vana miRNA Isolation Kit from Ambion.

Written steps are adapted from Ambion's manual for the *mir*Vana miRNA Isolation Kit and BioSpec's instructions for the Mini-BeadBeater-16.

# Materials

Equipment

Centrifuge

NAME

Benchtop Centrifuge

TYPE

Eppendorf

BRAND

5405000441

SKU

https://online-shop.eppendorf.us/US-en/Centrifugation-44533/Centrifuges-44534/Centrifuge-5425-PF-243560.html

LINK

Any benchtop centrifuge will suffice

SPECIFICATIONS

Equipment

Block heater

NAME

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BRAND

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SKU

Equipment

Vortex mixer

NAME

Any

BRAND

xx

SKU

Equipment

Mini-Beadbeater-16

NAME

high-energy cell disrupter

TYPE

BioSpec

BRAND

607

SKU

https://biospec.com/product/mini-beadbeater-16

LINK

1 speed

SPECIFICATIONS

Equipment

Fume hood

NAME

Fume hood

TYPE

Generic

BRAND

Unknown

SKU

Equipment

| | |
|---|---|
| Set of micropipettes with rack: 100-1000 µl, 20-200 µl, 2-20 µl, and 0.5-10 µl | NAME |
| Pipettor set | TYPE |
| Pipetman | BRAND |
| QP-1001-07 | SKU |
| https://www.minipcr.com/product/set-of-four-adjustable-volume-micropipettes-rack/ | LINK |
| Can use equivalent Pipettors | SPECIFICATIONS |

Equipment

| | |
|---|---|
| Bioanalyzer | NAME |
| Bioanalyzer | TYPE |
| Agilent | BRAND |
| G2991AA | SKU |
| https://www.agilent.com/en/product/bioanalyzer-automated-electrophoresis/bioanalyzer-instrument/2100-bioanalyzer-instrument-228250 | LINK |
| Any bioanalyzer will suffice. | SPECIFICATIONS |

- mirVanaTM miRNA isolation kit
- Ethanol Pure 200 proof for molecular biology **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**E7023-500mL
- Acid-Phenol:Chloroform, pH 4.5 (with IAA, 125:24:1) **Thermo Fisher Catalog #**AM9720
- Dry Ice
- DNA LoBind Tube 1.5ml **Eppendorf Catalog #**022431021
- Nuclease-free water, not DEPC-treated **Life Technologies Catalog #**AM9932
- Filter Tips
- Bioanalyzer RNA 6000 Nano Kit **Agilent Technologies Catalog #**5067

## Preparation

15m

1 Clean workspace, pipettes, and gloves with RNaseZAP.

2 Prepare bucket of ice.

3 Heat nuclease-free water to 95 °C.

4 Pre-cool microcentrifuge to 4 °C.

5 Add 200 proof pure ethanol to *mir*Vana wash buffers as instructed.

6 Pull frozen placenta samples (~150-200 mg each) from -80 °C and place on dry ice until ready to process.

Note

Integrity of isolated total RNA will be greater if tissue was preserved in RNA*later* as soon as possible after harvesting.

Typically, tissue is submerged in RNA*later* overnight. The following day, the RNA*later* is removed and the sample moved to -80 °C storage.

## Cell lysis and tissue disruption

20m

7 Add approximately 1 mL of 1.0mm zirconia/silica beads to each frozen placenta sample, and 700 µL *mir*Vana Lysis/Binding Buffer.

Note

After addition of beads and lysis buffer, tubes should be almost full. Exclude as much air as possible to reduce foaming.

Safety information

Samples should be in screw-cap microcentrifuge tubes with integral O-rings in the caps. Snap-cap tubes should not be used, unless secured with an adapter.

8 Load samples immediately into Mini-BeadBeater-16 vial holder ring. Up to 16 samples can be accommodated.

Safety information

IMPORTANT: Rotate the vial holder ring to a position where the small hole in the vial holder ring engages the anti-rotation pin sticking out of the wiggle mechanism. Slide the vial holder down the pin and seat it flat on the wiggle mechanism. Slide the large, black plastic hold-down cap over the stainless steel center bolt, aligning it so that it too slides down the anti-rotation pin. The hold-down cap must make contact with the top of the aluminum wiggle mechanism - not just the tops of the microcentrifuge tubes. Finally, screw on and hand-tighten the black knob firmly. Repeat: Tighten firmly.

9 Switch on the Mini-BeadBeater-16 and run for 00:02:00 . 2m

10 Place samples immediately into pre-chilled microcentrifuge and spin for 00:05:00 at maximum speed . 5m

11 Remove 500 µL lysate, being careful not to draw up particulates, and dispense into a fresh labeled microcentrifuge tube.

## Organic extraction 40m

12 Add 50 µL *mir*Vana miRNA Homogenate Additive (1:10 volume of original lysate) and vortex to mix. Incubate 00:10:00 On ice . 10m

13 Add 500 µL Acid-Phenol:Chloroform (1:1 volume of original lysate) and vortex 00:01:00 to homogenize sample. 1m

Note

Acid-Phenol:Chloroform may appear as a clear, homogeneous phenol phase (lower), overlayed by a small aqueous phase (upper). Pipette from the lower, not the upper, phase.

Safety information

Phenol is very corrosive and will severely burn the skin. Safety precautions such as gloves, protective eyewear, a lab coat, and working in a fume hood are critical. Discard contaminated pipette tips in appropriate waste container.

14 Spin 00:05:00 maximum speed in pre-chilled microcentrifuge. 5m

15 Carefully remove 350 µL of the top aqueous phase and transfer to a fresh labeled microcentrifuge tube.

Note

It is possible to remove a greater volume to maximize RNA yield, but make sure not to disturb the interphase or organic phase.

Safety information

Discard phenol liquid waste and contaminated tubes in appropriate waste containers.

## Total RNA isolation 30m

16 Add 437.5 µL (1.25 volumes) 200 proof pure ethanol and mix thoroughly.

17 Transfer up to 700 µL lysate/ethanol mixture to *mir*Vana Filter Cartridge (placed into *mir*Vana Collection Tube) and spin 10000 x g, 4°C, 00:00:15 in pre-chilled microcentrifuge. Discard flow-through. Repeat with remaining volume of lysate/ethanol mixture. 15s

Note

*mir*Vana Filter Cartridges can accommodate up to 700 µL volume - do not overfill. To avoid filter damage, do not spin at speeds greater than 10000 x g .

18 Add 700 µL *mir*Vana Wash Solution 1 and spin 10000 x g, 4°C, 00:00:15 in pre-chilled microcentrifuge. Discard flow-through. 15s

Note

Tip: flow-through can be aspirated into an appropriately-labeled waste container using a vacuum.

19 Add 500 µL *mir*Vana Wash Solution 2/3 and spin 10000 x g, 4°C, 00:00:15 in pre-chilled microcentrifuge. Discard flow-through. Repeat wash step. 15s

20 After discarding flow-through from the last step, spin 10000 x g, 4°C, 00:02:00 in pre-chilled microcentrifuge to remove residual Wash Solution. 2m

21 Transfer *mir*Vana Filter Cartridge into a fresh *mir*Vana Collection Tube. Add 40 µL pre-heated nuclease-free water to the center of the filter and close the cap. Incubate 00:01:00 and then spin 00:00:30 maximum speed in pre-chilled centrifuge to elute RNA. 1m 30s

22 Transfer eluate from the *mir*Vana Collection Tube to a low bind microcentrifuge tube and store at -80 °C .

Note

Minimize freeze/thaw cycles.

Tip: Store a small aliquot in a separate tube for QC.

## Quality control

1h 30m

23 Quantitate RNA using NanoDrop. Pay attention to A260/A280 ratio. For highly pure RNA, a ratio of 1.8-2.1 is expected. If necessary, repurify by adding 1/10th nuclease-free 5M NaCl and 1.38 volumes 200 proof pure ethanol before repassing the sample over a fresh *mir*Vana Filter Cartridge. Continue the total RNA isolation from Step 17.

Note

RNA can also be quantitated using Qubit RNA Broad Range Assay.

24 Assess RNA quality by running the RNA 6000 Nano Assay on an Agilent 2100 Bioanalyzer. A RIN score of >7.0 is normal for total RNA isolated from placenta.