

Aug 15, 2025

Version 1

miRNA extraction from fecal matter V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.kxygx43pdl8j/v1>

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Protocol Citation: nick Heyer 2025. miRNA extraction from fecal matter. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.kxygx43pdl8j/v1>

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Protocol status: In development

We are still developing and optimizing this protocol

Created: August 14, 2025

Last Modified: August 15, 2025

Protocol Integer ID: 224732

Keywords: mirna extraction from fecal matter, mirna extraction for tissue, mirna extraction, qiagen proticol, modification to the qiagen proticol, fecal matter, tissue

Disclaimer

This Proticol is still being eddited

Abstract

This is a modification to the Qiagen proticol for miRNA extraction for tissues



Prep

- 1 Set Centrifuge to 4C if needed
- 2 Create 70% and 90% Et-OH
 - 2.1 $\text{_____ num samples} \cdot 0.55 \cdot 0.7 = \text{_____ ml } 100\% \text{ Et-OH}$
 $\text{_____ num samples} \cdot 0.55 \cdot 0.3 = \text{_____ ml Molecular Grade Water}$
 - 2.2 $\text{_____ num samples} \cdot 0.55 \cdot 0.9 = \text{_____ ml } 100\% \text{ Et-OH}$
 $\text{_____ num samples} \cdot 0.55 \cdot 0.1 = \text{_____ ml Molecular Grade Water}$

Lysis, chloroform extraction, and Large Nucliotide removal

27m

- 3 In a 1.5ml tube, homogenize _____mg of dry fecal matter in 700ul of QIAzol 10m
- 4 Incubate at 20C for 5min
- 5 Add 140ul of chloroform, **MIX WELL** and let incubate for 1 min at 20C, **MIX AGAIN**, and incubate for 3 min
- 6 Centrifuge at >12,000xg at 4C for 15 min 15m
- 7 Transfer aqueous phase and determine volume by pipetting, combine with 1x Volume of 70% Et-OH . 2m
- 8 Run through a "RNeasy Mini spin Column" at >8,000xg at 20C



miRNA recovery

- 9 **Recover flow through**, and add 0.65x volumes for 100% Et-OH
- 10 Run through a "RNeasy Mini spin Column" at >8,000xg at 20C, 700ul at a time discarding flow-through
- 11 Wash matrix with 700ul RWT Buffer
- 12 Wash matrix with 500 ul of RPE
- 13 Wash matrix _____x with ice cold 90% Et-OH
- 14 Dry Tube by spinning column for 5 min
- 15 Elute in 30ul of elution buffer