Dec 26, 2025

m^{6}A RNA immunoprecipitation (MeRIP)

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzenmrvx1/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzenmrvx1/v1

Protocol Citation: Xin Xu, Yujuan Wang 2025. m6A RNA immunoprecipitation (MeRIP). **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzenmrvx1/v1

Protocol status: Working
We use this protocol and it's working

Created: December 22, 2025

Last Modified: December 26, 2025

Protocol Integer ID: 235602

Keywords: m6A, RNA methylation, MeRIP, MeRIP-seq, epitranscriptomics, RNA modification, RNA immunoprecipitation, cancer, transcriptome profiling, rna immunoprecipitation, enrichment of m6a, m6a, using fragmented total rna, total rna, fragmented total rna from cell line, modified rna, merip, antibody, purification for downstream library preparation, purification

Disclaimer

This protocol is provided for research use only and may require optimization depending on experimental conditions.

Abstract

This protocol describes m6A RNA immunoprecipitation (MeRIP) using fragmented total RNA from cell lines, followed by antibody-based enrichment of m6A-modified RNA and purification for downstream library preparation.

Guidelines

- Keep IGEPAL concentration consistent across buffers (0.1%) to minimize nonspecific binding while maintaining bead handling.
- During column cleanup, residual ethanol is a frequent cause of poor downstream enzymatic reactions; do the long dry spin.

Materials

Reagents and materials

Core reagents

- Total RNA (cell line), 5 µg
- **Protein A magnetic beads** (Thermo Fisher, **10002D**), 30 µL per IP**
- **Protein G magnetic beads** (Thermo Fisher, **10004D**), 30 µL per IP**
- **Anti-m6A antibody** (NEB, E1610S), 1 µL per IP (per your manuscript)
- **RNasin Plus RNase Inhibitor** (Promega, N2611)
- RNA Fragmentation Buffer (see recipe; your manuscript: 100 mM Tris-HCl pH 7.4; 100 mM ZnCl2)
- 0.5 M EDTA
- Ethanol (100% and 70%-75%)
- **RNeasy Mini Kit** (QIAGEN, **74106**) - for RLT and RPE****
- **RNeasy MinElute spin columns** (as used in your lab protocol for final cleanup)

meRIP-Seq Protocol

- Magnetic rack for 1.5 mL tubes
- End-over-end rotator (4°C)
- Thermomixer or rotator for washes (4°C)
- Optional QC: TapeStation High Sensitivity RNA ScreenTape

Buffers (prepare RNase-free)

**1x IP buffer (used for bead wash and IP)
- 10 mM Tris-HCl, pH 7.5
- 150 mM NaCl
- 0.1% IGEPAL CA-630

**5x IP buffer stock (optional but convenient for making the IP mixture)
Recipe example (30 mL): 4.5 mL 5 M NaCl + 1.5 mL 1 M Tris-HCl pH 7.5 + 0.15 mL IGEPAL + water to 30 mL.

1x low-salt wash buffer
- 10 mM Tris-HCl, pH 7.5
- 50 mM NaCl
- 0.1% IGEPAL CA-630

1x high-salt wash buffer
- 10 mM Tris-HCl, pH 7.5
- 500 mM NaCl
- 0.1% IGEPAL CA-630

Before start

- Use RNase-free tubes, tips, and reagents throughout.
- Perform all steps on ice or at 4°C unless otherwise indicated.
- Pre-chill rotators and magnetic racks.
- Prepare all buffers with nuclease-free water.
- Add RNase inhibitor immediately before use.

Procedure

1 Aliquot 5 µg total RNA into an RNase-free tube.
Adjust volume with nuclease-free water to 18 µL.

2 Add 2 µL RNA Fragmentation Buffer (final composition consistent with your manuscript: 100 mM Tris-HCl pH 7.4; 100 mM $ZnCl_2$).
Incubate at 70°C until theaverage size is ~180 nt (optimize time empirically for your setup).

3 Immediately place on ice. Add EDTA to chelate Zn^{2+} and stop fragmentation.

4 Ethanol precipitate the fragmented RNA, and resuspend fragmented RNA in RNase-free water.

5 Save 10% of fragmented RNA as input (store at -80°C).
Optional: assess fragmentation size distribution by TapeStation.

6 Vortex beads to resuspend.
Add 30 µL Protein A beads + 30 µL Protein G beads to a tube.

7 Add 1 mL cold 1x IP buffer, invert to mix, place on magnet, remove supernatant.
Repeat once (total 2 washes).

8 Resuspend beads in 500 µL 1x IP buffer.
Add 1 µL anti-m^6A antibody (NEB E1610S).
Rotate at 4°C for at least 6 h.

9 Wash the beads twice with 1 mL cold 1x IP buffer (magnet separate each time).
After the second wash, resuspend beads in 500 µL of IP reaction mixture (next section).

Immunoprecipitation (2 h at 4°C)

2h

10 In a final volume of 500 µL, combine:
Fragmented RNA (from Section A; excluding the 10% input)
100 µL 5x IP buffer (to achieve 1x in reaction)
5 µL RNasin Plus (Promega N2611)
Add nuclease-free water to 500 µL total
Add this mixture to the antibody-coupled beads.

11 Rotate 2 h at 4°C. 2h

Wash beads (stringent low/high-salt scheme) 1h 25m

12 Wash 1x IP buffer (2x) 25m
Add 1 mL cold 1x IP buffer, rotate 10 min at 4°C, magnet separate, discard supernatant.
Repeat once (total 2 washes).

13 Wash low-salt buffer (2x) 25m
Add 1 mL cold low-salt buffer, rotate 10 min at 4°C, magnet separate, discard supernatant.
Repeat once.

14 Wash high-salt buffer (2x) 25m
Add 1 mL cold high-salt buffer, rotate 10 min at 4°C, magnet separate, discard supernatant.
Repeat once.

15 Final quick spin (optional) 10m
Briefly spin tube (5-10 s) to collect residual liquid.
Place on magnet and remove remaining wash buffer carefully.

Elution and RNA purification (RNeasy MiniElute) 22m

16 Elute from beads with RLT 5m
Add 200 µL RLT buffer directly to beads.
Mix and incubate 2 min at room temperature.
Place on magnet and transfer supernatant to a new tube.

17 Bind to column 5m
Add 400 µL 100% ethanol to the eluate, mix well.
Load onto an RNeasy MiniElute spin column; centrifuge at 12,000 rpm, 1 min, 4°C.
If needed, load in multiple passes until the full volume is processed.

18 Wash column 10m
Wash with 500 µL RPE once.
Wash with 500 µL 80% ethanol once.
Spin full speed 5 min at 4°C to remove residual ethanol.

19 Elute IP RNA 2m
Elute with 14 µL ultrapure H2O.
Store at -80°C.

Optional checkpoints (recommended)

4h

20 Fragmentation QC: TapeStation HS RNA to confirm ~180-200 nt distribution.
IP efficiency QC: RT-qPCR for known methylated vs negative regions.

4h

*

Notes and common pitfalls

21 Fragmentation time is the main driver of final fragment size. Once you have a time that yields ~180 nt for 5 µg in your thermocycler, keep it constant across samples.
Keep IGEPAL concentration consistent across buffers (0.1%) to minimize nonspecific binding while maintaining bead handling.
During column cleanup, residual ethanol is a frequent cause of poor downstream enzymatic reactions; do the long dry spin.

Protocol references

Zeng, Y. et al. Refined RIP-seq protocol for epitranscriptome analysis with low input materials. PLoS Biol. 16, e2006092 (2018).