EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit

Base Catalog # P-9018

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit is designed to enrich an RNA fragment containing m^6A from low input RNA and to identigy region-specific m^6A by PCR or profile epitranscriptome-wide m^6A by next generation sequencing using Illumina platforms or other methods. The innovative working principle, optimized protocol, and components of the kit allow for the capture of the m6A fragment with minimal non-specific background levels. The enriched RNA is specifically suitable to construct both non-barcoded (singleplexed) and barcoded (multiplexed) libraries quickly, allowing m6A regions to be mapped with less bias and at a high resolution.

Input Amount: In general, the amount of total RNA for each reaction can range from 1 µg to 20 µg. For optimal preparation, the input amount should be 10 µg RNA, although data can be obtained from as low as 500 ng of total RNA using this kit.

Starting Materials: Starting materials can include various mammalian cell samples such as culture cells from a flask or plate, primary cells or rare cell populations isolated from blood, body fluid and fresh/frozen tissues, specific cells sorted from entire cell population and embryonic cells, etc.

Antibodies: The anti-m6A rabbit polyclonal antibody used in this kit is highly specific against m^6A RNA fragments, MeRIP-grade, and not cross-reactive to adenine-unmethylated RNA fragments.

Internal Controls: A positive control is provided in this kit.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the strip wells. Use aerosol-barrier pipette tips and always change pipette tips between liquid transfers. Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

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KIT CONTENTS

Component	24 reactions Cat. #P-9018-24	Storage Upon Receipt
WB (Wash Buffer)	30 ml	4°C
ICB (Immuno Capture Buffer)	5 ml	RT
NDE (Nuclear Digestion Enhancer)	300 µl	RT
CEM (Cleavage Enzyme Mix)*	60 µl	-20°C
m6A Antibody (1 mg/ml)*	50 µl	-20°C
Non-Immune IgG (1 mg/ml)*	20 µl	4°C
m^{6}A-Positive Control (200 µg/ml)**	6 µl	-20°C
PDB (Protein Digestion Buffer)	5 ml	RT
Proteinase K (10 mg/ml)*	100 µl	4°C
Affinity Beads*	100 µl	4°C
RPS (RNA Purification Solution)	600 µl	RT
RNA Binding Beads*	60 µl	4°C
Elution Buffer	2000 µl	RT

** Spin the solution down to the bottom prior to use.*
*** m^{6}A-Positive Control is a synthesized oligo containing m^{6}A*

SHIPPING & STORAGE

The kit is shipped in two parts: the first part at ambient room temperature and the second part on frozen ice packs at 4°C.

Upon receipt, store the components according to the temperatures in the table above away from light. The kit can be stable for up to 6-months from the date of shipment when stored properly.

Note: *Check if **WB (Wash Buffer)** contains salt precipitates before use. If so, briefly warm at room temperature or 37°C and shake the buffer until the salts are re-dissolved.*

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Vortex mixer
- ☐ Thermocycler with 48 or 96-well block
- ☐ microplate rotator or rolling shaker
- ☐ Magnetic device (96-well PCR plate format)
- ☐ Adjustable pipette and pipette tips
- ☐ 0.2 ml or 0.5 ml PCR vials

- ☐ 1.5 ml microcentrifuge tubes
- ☐ RNA sample
- ☐ 100% ethanol
- ☐ Distilled water
- ☐ 1X TE buffer

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit is tested against predetermined specifications to ensure consistent product quality. EpigenTek guarantees the performance of all products in the manner described in our product instructions.

Product Warranty: If this product does not meet your expectations, simply contact our technical support unit or your regional distributor. We also encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

Safety: Suitable lab coat, disposable gloves, and proper eye protection are required when working with this product.

Product Updates: EpigenTek reserves the right to change or modify any product to enhance its performance and design. The information in this User Guide is subject to change at any time without notice. Be sure to use the latest User Guide for this kit which can be accessed online at www.epigentek.com/datasheet.

Usage Limitation: The EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit is for research use only and is not intended for diagnostic or therapeutic application.

Intellectual Property: The EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

N6-methyl-adenosine (m6A) is the most common and abundant modification on RNA molecules present in eukaryotes. The m6A modification is catalyzed by a methyltransferase complex METTL3 and removed by m6A RNA demethylases FTO and ALKBH5, which catalyze m6A demethylation in an α-ketoglutarate (α-KG)- and Fe2+-dependent manner. METTL3, FTO, and ALKBH5 are known to play important roles in many biological processes, ranging from development and metabolism to fertility. m6A accounts for more than 80% of all RNA base methylations and exists in various species. m6A is mainly distributed in mRNA and also occurs in non-coding RNA, such as tRNA, rRNA, and snRNA. The relative abundance of m6A in mRNA transcripts has been shown to affect RNA metabolism processes such as splicing, nuclear export, translation ability and stability, and RNA transcription. Abnormal m6A methylation levels induced by defects in m6A RNA methylase and demethylase could lead to dysfunction of RNA and cause disease. For example, abnormally low levels of m6A in target mRNAs due to increased FTO activity in patients with FTO mutations, through an as-yet-undefined pathway, contributes to the onset of obesity and related diseases. The dynamic and reversible chemical m6A modification on RNA may also serve as a novel epigenetic marker of profound biological significance. Therefore, more useful information for a better understanding of m6A RNA

methylation levels and distribution on RNA transcripts could benefit diagnostics and therapeutics of disease.

Currently, several methods are used for epitranscriptome-wide m6A mapping. These methods include MeRIP, PA-m6A-seq, miCLIP, and m6A-CLIP. MeRIP has been widely used but is unable to achieve high resolution in m6A profiling. PA-m6A-seq, miCLIP, and m6A-CLIP improve the profiling resolution but suffer from poor reproducibility and a complicated process. In particular, these methods are time-consuming (>2 days) and costly. To address these issues, EpigenTek has developed a new method: CUT&RUN M6A Enrichment (cleavage under target and recover using nuclease for m6A enrichment). Our innovative approach combines the advantages of MeRIP and m6A-CLIP with the fastest procedures and incorporates it into the EpiQuik CUT&RUN m6A RNA Enrichment Kit. This kit has the following features:

- **High enrichment:** Uses an RNA cleavage enzyme mix to simultaneously fragment RNA and cleave/remove any RNA sequences in both ends of the target m6A-containing sequences without affecting RNA regions occupied by the antibody. Short RNA fragments are generated only bound with anti- m6A antibody. True target m6A-enriched regions can therefore be reliably identified, and high-resolution mapping achieved.
- **Low input:** Unbound RNA cleavage and immunocapture are processed in the same single-tube, which enables maximum protection of the target m^6A-containing regions and minimized sample loss, allowing the input RNA to be as low as 500 ng.
- **Minimal Background:** Cleavage of unbound RNA sequences in the two (2) ends of the target m^6A-containing sequences enables minimized MeRIP/sequencing background, allowing data analysis with <10 million reads.
- **Fast, streamlined procedure:** The procedure from RNA to enriched m6A RNA is around 2 hours and 30 min with <30 min of hands-on time
- **Highly convenient:** The kit contains all required components for each step of the EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit, which are sufficient for m^6A-containing RNA sequence capture, thereby allowing m^6A RNA enrichment to be the most convenient with reliable and consistent results.

PRINCIPLE & PROCEDURE

The EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit contains all necessary reagents required for carrying out a successful m^6A RNA enrichment starting from total RNA. In the reaction, RNA sequences in both ends of the target m^6A-containing regions are cleaved/removed, and the target m^6A-containing fragments are pulled down using a beads-bound m^6A capture antibody. The enriched RNA is then released, purified, and eluted. Included in the kit are a non-immune IgG control and m^6A positive control. These can be used to demonstrate the efficacy of the kit and performance at the enriched RNA quantification or bioanalyzer step.

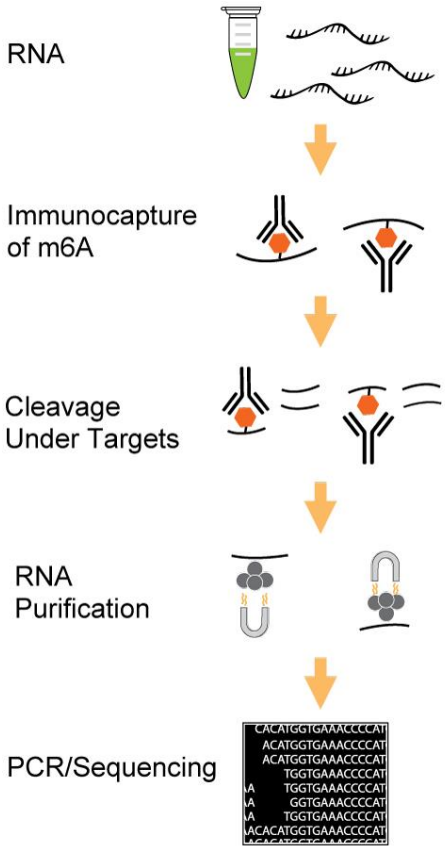

Fig. 1. Workflow of the EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit.

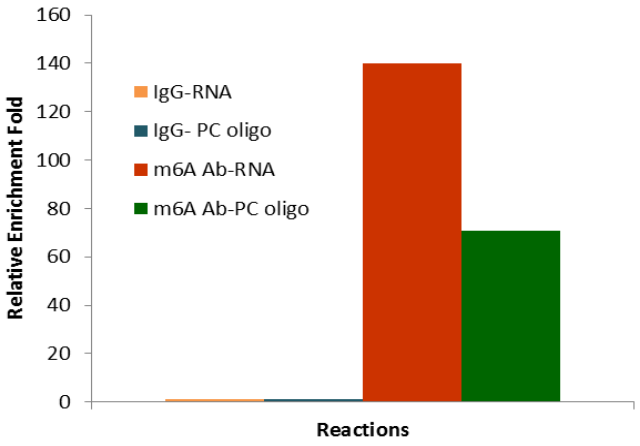

Fig 2. m^{6}A RNA enrichment using the EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit: RNA fragments containing m^{6}A were captured by the anti- m^{6}A antibody (#A-1801, EpigenTek) from 10 µg of total human RNA and 500 ng of the positive control oligo, respectively. Non-immune IgG was used as a negative control. The enriched RNA was purified and fluorescently quantified for enrichment fold comparison.

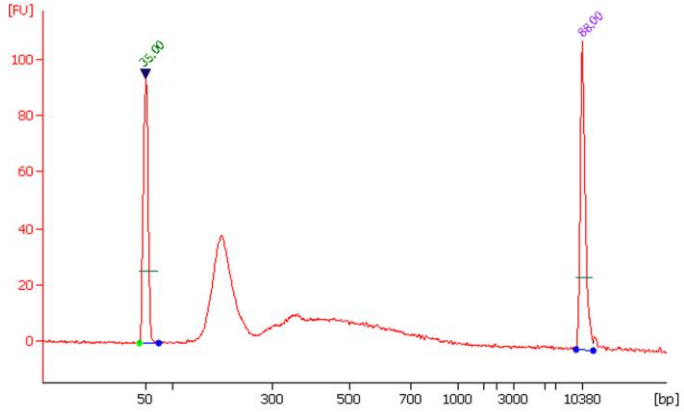

Fig 3. Size distribution of library fragments: m^{6}A fragments were enriched from 5 µg of total human RNA by the anti- m^{6}A antibody (#A-1801, EpigenTek,) and used for cDNA library preparation. Peak at 195 bps reflects insert size (around 55 bps) of RNA bound by m^{6}A antibody.

Procedure Overview & Estimated Time Table:

Steps	Approx. Required Time
m6A capture and cleavage	100 min
Enriched RNA release and purification	40 min*

ASSAY PROTOCOL

For the best results, please read the protocol in its entirety prior to starting your experiment.

Starting Materials

Input RNA Amount: Total RNA amount can range from 1 µg to 20 µg per reaction. An optimal amount is 10 µg per reaction, as m^6A fraction is generally less than 0.1% of total RNA. Starting RNA may be in water or in a buffer such as TE. RNA should be high quality and relatively free of DNA. DNAse can be used to remove DNA, and RNA should be eluted in RNase-free water.

RNA Storage: RNA should be stored at -20°C or -80°C until use.

1. Immunocapture and Cleavage

a. Prepare immunocapture solution by adding the reagents to 0.2 ml PCR tubes according to the following and mix well:

Reagents	Sample	Non-Immune IgG	Positive Control
ICB (**Immuno Capture Buffer**)	174-189 µl	174-189 µl	191 µl
m6A antibody	2 µl	0	2 µl
RNA sample	5-20 µl	5-20 µl	0
Non-Immune IgG	0	2 µl	0
Positive control oligo	0	0	3 µl
Affinity Beads	4 µl	4 µl	4 µl
Total Volume	**200 µl**	**200 µl**	**200 µl**

Note: *1) The final amount of each component should be (a) antibodies of interest: 2 µg/tube and (b) non-immune IgG: 2 µg/tube. 2) Shake the* ***Affinity Beads*** *to completely suspend them before use.*

b. Rotate the tube on a rotator or rolling shaker at room temperature for 90 min.

c. After the 90 min incubation, add 10 µl of **NDE (Nuclear Digestion Enhancer),** 2 µl of **CEM (Cleavage Enzyme Mix)** to each tube and incubate at room temperature for 4 min.

d. Put the tubes on the magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the RNA).*

The Input can be cleaved according to the following steps:

Add 1-2 µl (200-400 ng) of the RNA into 20 µl of **ICB** (**Immuno Capture Buffer)** followed by adding 1.5 µl of **NDE (Nuclear Digestion Enhancer),** 1 µl of **CEM** (**Cleavage Enzyme Mix**) and incubate at room temperature for 2 min. Then go to step 2d for RNA release/recovery.

e. Keep the PCR tubes in the magnetic stand and wash each reaction tube three times with 150 µl of **WB (Wash Buffer),** and once with 150 µl of **PDB (Protein Digestion Buffer**). The wash can be performed as follows:

After the solution has been removed, add **WB (Wash Buffer)** to the reaction tubes. Resuspend the beads by gently pipetting up and down several times. Ensure the beads are completely resuspended and the beads are not clinging to the pipette tips after pipetting. Place the tubes back in the magnetic stand for 1-2 min to pellet the beads and then remove and discard the solution from each reaction tube.

2. Enriched RNA Release/Recovery

a. Prepare **Protein Digestion Solution** by mixing **Proteinase K** with **PDB** (**Protein Digestion Buffer**) at 1:10 (e.g., 1 µl of **Proteinase K** + 9 µl of **PDB (Protein Digestion Buffer**).

b. Remove the tubes from the magnetic device after the last wash. Add 20 µl of **Protein Digestion Solution** to each sample and negative control. Mix and incubate at 55°C for 15 min in a thermocycler (without heated lid).

c. Put the tubes on the magnetic device until the solution is clear (about 2 min). Carefully transfer the solution from each sample to an unused PCR tube.

d. Add 20 µl of the **RPS (RNA Purification Solution)** into each sample and the negative tubes followed by adding 160 µl of 100% Ethanol. And 25 µl of the **RPS (RNA Purification Solution)** into Input tube followed by add 200 µl of 100% Ethanol.

e. Resuspend **RNA Binding Beads** by vortexing. Add 2 µl of the resuspended beads to each tube. Mix thoroughly by pipetting up and down at least 10 times.

f. Incubate for 5 min at room temperature to allow the RNA to bind to the beads.

g. Put the PCR tubes in the magnetic device until the solution is clear (about 2 min). Carefully remove and discard the supernatant. *(Caution: Be careful not to disturb or discard the beads that contain the RNA).*

h. Keep the PCR tubes in the magnetic device and add 150 µl of freshly prepared *90% ethanol* to the tubes, then carefully remove and discard the ethanol.

i. Repeat Step 2 h once for a total of two washes.

j. Resuspend the beads in 13 µl of **Elution Buffer** and incubate at room temperature for 5 min to release the RNA from the beads.

k. Capture the beads by placing the tubes in the magnetic device until the solution is completely clear (about 1 min).

l. Transfer 13 µl from each sample to a new 0.2 ml PCR tube for immediate use or store at -20C.

Note: *(1) The concentration of the enriched RNA can be simply quantified by a fluorescence method to know the enrichment fold with comparison between the positive control and the negative control or between the sample and the IgG control. For example, take 1 ul of eluted RNA for quantification with use of RNA or ssDNA quantification methods; (2) For use of the enriched RNA to construct library for sequencing, you can use your own successful RNA library construction methods or use the RNA library preparation kits available from Illumina or NEB.*

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Little or no enriched RNA generated from sample.	Insufficient amount of qualified RNA or m^6A-contiaing RNA.	Use higher amount of RNA.
	Poor enrichment with antibody.	Check if the immunocapture condition is correct.
	Inappropriate RNA fragmenting condition.	**CEM (Cleavage Enzyme Mix)** may be degraded due to improper temperature storage. Ensure the proper storage conditions for this component. **CEM (Cleavage Enzyme Mix)** reaction time is too short or too long. The cleavage conditions should be optimized to allow RNA fragment size to be between 30-200 bps.
	Incorrect temperature and/or insufficient time during RNA release.	Ensure that proper incubation times and temperatures described in the protocol are followed correctly.
	Improper reaction conditions at each reaction step.	Check if the reagents are properly added and incubation temperature and time are correct at each reaction step including RT reaction, library synthesis, and Amplification.
	Improper storage of the kit.	Ensure that the kit has not exceeded the expiration date. Standard shelf life, when stored properly, is 6 months from date of receipt.
No difference in enriched RNA intensity between negative control and the samples	Insufficient washing.	Check if washing recommendations at each step is performed according to the protocol. If the signal intensity in the negative control is still high, washing stringency can be increased in the following ways: 1. Increase wash time at each wash step: after adding **WB (Wash Buffer)**, leave it in the wells for 3-4 min and then remove it. 2. Add an additional one wash with **WB (Wash Buffer),** respectively: The provided volume of **WB (Wash Buffer)** is sufficient for 4 extra washes for each sample.

RELATED PRODUCTS

RNA Methylation Quantification

P-9003	Methylamp™ RNA Bisulfite Conversion Kit
P-9005	EpiQuik™ m^6A RNA Methylation Quantification Kit (Colorimetric)
P-9008	EpiQuik™ m^6A RNA Methylation Quantification Kit (Fluorometric)
P-9010	MethylFlash™ m6A DNA Methylation ELISA Kit (Colorimetric)
P-9015	MethylFlash™ Urine N6-methyladenosine (m6A) Quantification Kit (Colorimetric)
P-9016	EpiNext™ CUT&RUN RNA m6A-Seq Kit

DNA Isolation and Cleanup

P-1003	FitAmp™ General Tissue Section DNA Isolation Kit
P-1004	FitAmp™ Plasma/Serum DNA Isolation Kit
P-1006	DNA Concentrator Kit
P-1009	FitAmp™ Paraffin Tissue Section DNA Isolation Kit
P-1017	FitAmp™ Urine DNA Isolation Kit
P-1018	FitAmp™ Blood and Cultured Cell DNA Extraction Kit

DNA Enrichment Reaction

P-1015	Methylamp™ Methylated DNA Capture (MeDIP) Kit
P-1038	EpiQuik™ Hydroxymethylated DNA Immunoprecipitation (hMeDIP) Kit
P-1052	EpiQuik™ MeDIP Ultra Kit
P-2002	EpiQuik™ Chromatin Immunoprecipitation (ChIP) Kit
P-2003	EpiQuik™ Tissue Chromatin Immunoprecipitation (ChIP) Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2025	ChromaFlash™ One-Step ChIP Kit
P-2026	ChromaFlash™ One-Step Magnetic ChIP kit
P-2027	ChromaFlash™ ChIP Ultra Kit
P-2030	EpiNext™ ChIP-Seq High Sensitivity Kit

PCR Analysis

P-1029	EpiQuik™ Quantitative PCR Kit

DNA Library Prep

P-1051	EpiNext™ DNA Library Preparation Kit (Illumina)
P-1053	EpiNext™ High-Sensitivity DNA Library Preparation Kit (Illumina)

NGS Barcode

P-1060	EpiNext™ NGS Barcode (Index) Set-12

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