EpiNext™ CUT&RUN RNA m6A-Seq Kit

Base Catalog # P-9016

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The EpiNext™ CUT&RUN RNA m6A-Seq Kit is designed to enrich an RNA fragment containing m^6A from low input RNA and to prepare a library for next generation sequencing using Illumina platforms such as Illumina Genome Analyzer II, HiSeq and MiSeq systems. 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 for the construction of 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 be 1 µg to 20 µg. For optimal preparation, the input amount should be 10 µg RNA, although this CUT&RUN RNA m^6A -Seq data can be obtained with an amount of total RNA as low as 500 ng.

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 with MeRIP-grade, and is not cross-reactive to adenine-unmethylated RNA fragments.

Internal Controls: A negative control non-immune IgG is provided in this kit.

Precautions: To avoid cross-contamination, carefully pipette the sample or solution into the tubes. 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

For m6A Capture Reaction

Component	12 reactions Cat. #P-9016-12	24 reactions Cat. #P-9016-24	Storage Upon Receipt
WB (Wash Buffer)	15 ml	30 ml	4°C
CB (Capture Buffer)	2 ml	4 ml	RT
NDE (Nuclear Digestion Enhancer)	150 µl	300 µl	RT
CEM (Cleavage Enzyme Mix)*	30 µl	60 µl	-20°C
M^{6}A Antibody (1 mg/ml)*	25 µl	50 µl	-20°C
Non-Immune IgG (1 mg/ml)*	10 µl	20 µl	4°C
PDB (Protein Digestion Buffer)	2.5 ml	5 ml	RT
Proteinase K (10 mg/ml)*	50 µl	100 µl	4°C
Affinity Beads*	50 µl	100 µl	4°C
RPS (RNA Purification Solution)	300 µl	600 µl	RT
NA Binding Beads*	30 µl	60 µl	4°C
Elution Buffer	1 ml	2 ml	RT

For Library Preparation

Component	12 reactions Cat. #P-9016-12	24 reactions Cat. #P-9016-24	Storage Upon Receipt
5X Reaction Buffer*	100 µl	200 µl	-20ºC
RT Enzyme Mix*	13 µl	26 µl	-20°C
Adaptor-A (10 µM)*	28 µl	56 µl	-20°C
Reaction Enzyme Mix*	25 µl	50 µl	-20°C
Adaptor-B (10 µM)*	28 µl	56 µl	-20°C
MQ Binding Beads*	1.8 ml	3.6 ml	4°C
2X HiFi PCR Master Mix*	160 µl	320 µl	-20°C
Primer U (10 µM)*	15 µl	30 µl	-20°C
Primer I (10 µM)*	15 µl	30 µl	-20°C

** Spin the solution down to the bottom prior to use.*

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
- ☐ Agilent® Bioanalyzer® or comparable method to assess the quality of DNA library
- ☐ 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
- ☐ 90% ethanol (freshly prepared)
- ☐ Distilled water
- ☐ 1X TE buffer

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the EpiNext™ CUT&RUN RNA m6A-Seq 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 EpiNext™ CUT&RUN RNA m6A-Seq Kit is for research use only and is not intended for diagnostic or therapeutic application.
Intellectual Property: The EpiNext™ CUT&RUN RNA m6A-Seq Kit and methods of use contain proprietary technologies by EpigenTek.

A BRIEF OVERVIEW

N^6-methyl-adenosine (m^6A) is the most common and abundant modification on RNA molecules present in eukaryotes. The m^6A modification is catalyzed by a methyltransferase complex METTL3 and removed by m^6A RNA demethylases FTO and ALKBH5, which catalyze m^6A demethylation in an α-ketoglutarate (α-KG)- and Fe^{2+}-dependent manner. METTL3, FTO and ALKBH5 are known to play important roles in many biological processes, ranging from development and metabolism to fertility. m^6A accounts for more than 80% of all RNA base methylations and exists in various species. m^6A is mainly distributed in mRNA and also occurs in non-coding RNA, such as tRNA, rRNA and snRNA. The relative abundance of m^6A in mRNA transcripts has been shown to affect RNA metabolism processes such as splicing, nuclear export, translation ability and stability and RNA transcription. Abnormal m^6A methylation levels induced by defects in m^6A RNA methylase and demethylase could lead to dysfunction of RNA and cause disease. For example, abnormally low levels of m^6A 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 m^6A modification on RNA may also serve as a novel epigenetic marker of profound biological significance. Therefore, more useful information for better understanding of m^6A RNA methylation levels and distribution on RNA transcripts could benefit diagnostics and therapeutics of disease.

Currently, there are several methods that are used for epitranscriptome-wide m^6A mapping. These methods include MeRIP-seq, PA-m^6A-seq, miCLIP, and m^6A-CLIP. MeRIP seq has been widely used but is unable to achieve high resolution in m^6A profiling. PA-m^6A-seq, miCLIP, and m^6A-CLIP improve the profiling resolution but suffer from poor reproducibility and 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 RNA m^6A –Seq (cleavage under target and recover using nuclease for m^6A sequencing). Our innovative approach combines the advantages of MeRIP and m6A-CLIP with the fastest procedures, and incorporates it into EpiNext™ CUT&RUN RNA m6A-Seq Kit. This kit has the following features:

- **High Enrichment:** Use RNA cleavage enzyme mix to simultaneously fragment RNA and cleave/remove any RNA sequences in the both end of the target m^6A -containing sequences without affecting RNA regions occupied by the antibody. Short RNA fragments are generated only bound with anti- m^6A antibody. True target m^6A-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 the maximal protection of the target m^6A-containing regions and the minimized sample loss, allowing the input RNA to be as low as 500 ng.
- **Minimal Background:** Cleavage of unbound RNA sequences in the two end of the target m^6A-containing sequences enables the minimized MeRIP/sequencing background, allowing data analysis with <10 million reads
- **Fast, streamlined procedure:** The procedure from RNA to library cDNA is less than 6 hours with <1 h of hands-on time
- **Highly convenient:** The kit contains all required components for each step of the CUT&RUN RNA m6A -Seq, which are sufficient for both m^6A-containing RNA sequence capture and captured cDNA library preparation, thereby allowing CUT&RUN RNA m^6A –Seq to be the most convenient with reliable and consistent results.

PRINCIPLE & PROCEDURE

The EpiNext™ CUT&RUN RNA m6A-Seq Kit contains all necessary reagents required for carrying out a successful m^{6}A-Seq starting from total RNA. In the reaction, RNA sequences in both ends of the target m^{6}A-containing regions are cleaved/removed and the target m^{6}A-containing fragments are pulled down using beads-bound m^{6}A capture antibody. The target m^{6}A-containing RNA fragments are then recovered, released, reverse-transcripted, and adaptor ligated. The ligated first strand cDNA is amplified, and then used as template for library synthesis. A high-fidelity PCR mix is used for library cDNA amplification and indexing. The cDNA is then cleaned up using binding beads. Included in the kit is a negative control non-immune IgG, which can be used to demonstrate the efficacy of the kit and performance at the enriched RNA quantification or bioanalyzer analysis step.

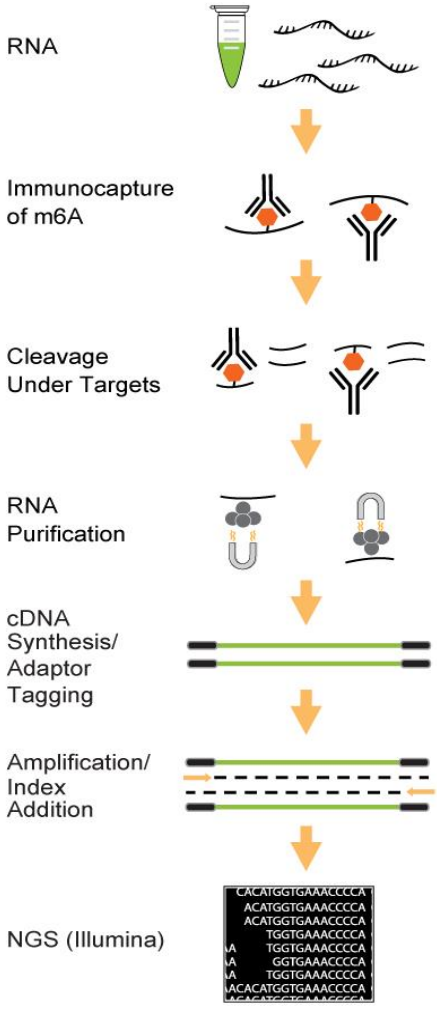

Fig 1. Workflow of the EpiNext™ CUT&RUN RNA m6A-Seq Kit.

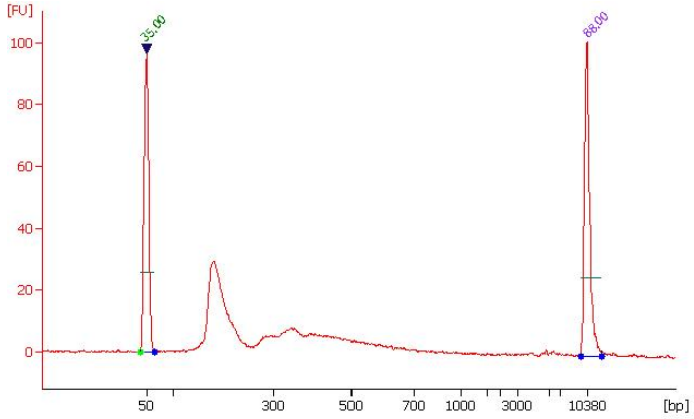

Fig 2. Size distribution of library fragments. m^{6}A fragments were enriched from 3 ug of total RNA by the anti- m^{6}A antibody (EpigenTek, #A-1801) and used for cDNA library preparation. Peak at 195 bps reflects insert size (around 55 bps) of RNA bound by m^{6}A antibody.

Procedure overview and time Table

Steps	Required time
m^{6}A capture and cleavage	100 min
Reverse-transcription and ligation	150 min
Ligated cDNA release and purification*	40 min*
Library amplification and purification	50 min

**Stop point. cDNA generated at this step can be stored at -20°C for future amplification use*

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 m6A 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 I 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 (long term) 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	Negative Control
CB (Capture Buffer)	174-189 µl	174-189 µl
m^{6}A antibody	2 µl	0
RNA sample	5-20 µl	5-20 µl
Non-Immune IgG	0	2 µl
Affinity Beads	4 µl	4 µl
Total Volume	**200 µl**	**200 µl**

Note: *1). The final amount of each component should be (a) m6A antibodies 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 5 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 DNA).*

The Input (un-ChIPed control) can be cleaved according to the following steps:

Add 1-2 µl (200-400 ng) of the RNA into 20 µl of **CB (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 and 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 pellets 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 **NA 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 2h 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.

Optionally, if the input amount of RNA is >10 µg, the enriched RNA could be quantified. For example, take 1 ul of eluted RNA for quantification with use of RNA or ssDNA quantification method. The same volume of elution from IgG negative control could be used for determining the enrichment fold.

3. First Strand cDNA Synthesis

a. Prepare the first strand cDNA synthesis reaction in 0.2 ml PCR tube according to Table 1:

Table 1. First Strand cDNA Conversion

Component	Volume
Enriched RNA (from Step 2) *	13 µl
5X Reaction Buffer	4 µl
Adaptor-A (10 µM)	2 µl
RT Enzyme Mix	1 µl
Total volume	**20 µl**

***** *If the enriched RNA volume is less than 13 µl, add distilled water to make the total volume 20 µl.*

b. Mix and incubate for 50 min at 42°C in a thermocycler without heated lid (make sure to set lid temperature to 25°C).

4. Clean-Up of the first strand cDNA

***Note**: To ensure the correct ratio of **MQ Binding Beads** to sample solution during DNA clean up , make sure that any bead solution stuck on the outside of the pipette tip is removed before adding beads into the sample vial.*

a. Resuspend **MQ Binding Beads** by vortex.
b. Add exactly 50 µl of resuspended beads to the PCR tube of cDNA synthesis reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain cDNA.
e. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Repeat Step 4e two times for a total of three washes.

g. Open the cap of the PCR tube and air dry beads for 2-3 minutes while the tube is on the magnetic stand.
h. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.
i. Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.
j. Transfer 12 µl of clear solution to a new 0.2 ml PCR tube for library synthesis.

5. Library Synthesis

a. Prepare library synthesis reaction in a 0.2 ml PCR tube according to Table 2:

Table 2. Library Synthesis

Component	Volume
First strand cDNA (from Step 4)	12 µl
5X Reaction Buffer	4 µl
Adaptor-B (10 µM)	2 µl
Total volume	**18 µl**

b. Mix and incubate for 2 min at 98°C in a thermocycler without heated lid (make sure to set lid temperature to 25°C) followed by incubation on ice for 2 min. Add 2 µl of **Reaction Enzyme Mix** and then incubate at 37°C for 60 min in a thermocycler without heated lid.

6. Clean-Up of Synthesized Library

a. Resuspend **MQ Binding Beads** by vortex.
b. Add exactly 36 µl of resuspended beads to the tube of library synthesis reaction. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.
c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.
d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.
e. Remove tube from magnet. Add 150 µl of freshly prepared *90% ethanol* to the tube to resuspend the beads. Put the PCR tube back in the magnetic stand. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
f. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.
g. Repeat Step 6f one more time for a total of three washes.
h. Open the cap of the PCR tube and air dry beads for 2-3 minutes while the tube is on the magnetic stand.
i. Resuspend the beads in 12 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.

j. Capture the beads by placing the tube in the magnetic stand for 2 minutes or until the solution is completely clear.

k. Transfer 10.5 µl of clear solution to a new 0.2 ml PCR tube for library amplification and indexing.

Note: *Take 1* µl *of the eluted DNA and quantify the concentration of the synthesized cDNA by a fluorescence method so that the library amplification cycles can be determined at Step 7 of library amplification. We recommend using our FitAmp™ General DNA Quantification Kit (#P-1020, EpigenTek) for the synthesized cDNA quantification.*

7. Library Amplification and Indexing

a. Prepare the PCR Reactions:

Thaw all reaction components including master mix and primer solution. Mix well by vortexing briefly. Keep components on ice while in use, and return to –20˚C immediately following use. Add components into each PCR tube/well according to Table 3:

Table 3. Library Amplification and Indexing

Component	Size (µl)
HiFi Master Mix (2X)	12.5 µl
Primer U	1 µl
Primer I (or barcode index)	1 µl
Synthesized library DNA (from Step 6)	10.5 µl
Total Volume	**25 µl**

Important Note: (1) *Use of* ***Primer I*** *included in the kit will generate a singleplexed library*. *For multiplexed library preparation, replace* ***Primer I*** *with one of the12 different barcodes (indexes) contained in the EpiNext™ NGS Barcode (Index) Set-12 (Cat# P-1060), EpigenTek) to generate each indexed library. You can also add user-defined barcodes (Illumina compatible) instead of* ***Primer I****. (2) Each indexed library can be combined in equal amounts to form multiplexed libraries for sequencing. (3) The amount of indexed library can be quantified using qPCR, Qubit or Picogreen assays.*

b. Program the PCR Reactions:

Place the reaction plate in the instrument and set the PCR conditions as follow:

Cycle Step	Temp	Time	Cycle
Activation	98°C	30 sec	1
Cycling	98°C 55°C 72°C	10 sec 20 sec 20 sec	varied*
Final Extension	72°C	2 min	1

** PCR cycles may vary depending on the ligated DNA concentration. In general, use 14 cycles for 8 ng, 17 cycles for 2 ng, and 21 cycles for 0.5 ng of the ligated DNA. Further optimization of PCR cycle number may be required.*

8. Clean-Up of Amplified Library

a. Resuspend **MQ Binding Beads** by vortex.

b. Add exactly 20 µl (0.8X) of resuspended beads to the amplified library. Mix thoroughly on a vortex mixer or by pipetting up and down at least 10 times.

c. Incubate for 5 minutes at room temperature to allow DNA to bind to beads.

d. Put the PCR tube on an appropriate magnetic stand until the solution is clear (about 2-3 minutes). Carefully remove and discard the supernatant. Be careful not to disturb or discard the beads that contain DNA.

e. Keep the PCR tube in the magnetic stand and add 150 µl of freshly prepared *90% ethanol* to the tube. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

f. Remove tube from magnet. Add 150 µl of freshly prepared *90% ethanol* to the tube to resuspend the beads. Put the PCR tube back in the magnetic stand. Incubate at room temperature for 1 min, and then carefully remove and discard the ethanol.

g. Repeat Step 8f one more time for a total of three washes.

h. Open the PCR tube cap and air dry beads for 2-3 minutes while the tube is on the magnetic stand.

i. Resuspend the beads in 10 µl **Elution Buffer**, and incubate at room temperature for 2 minutes to release the DNA from the beads.

j. Capture the beads by placing the tube in the magnetic stand for 2-3 minutes or until the solution is completely clear.

k. Transfer 10 µl to a new 0.2 ml PCR tube for immediate use or store at -20°C until ready to use for sequencing.

Note: *(1) Quality of the prepared library can be assessed using an Agilent® Bioanalyzer® or comparable method. Library fragments should have the correct size distribution (ex: 200-300 bps at peak size) without adaptors or adaptor-dimers (about 130 bps). (2) To check the size distribution, dilute library with water (if necessary) and apply it to an Agilent high sensitivity chip. If there is the presence of <150 bp adaptor dimers, it is recommended to use 0.9X* ***MQ Binding Beads*** *to remove fragments below 150 bps. (3) The amount of indexed library can be quantified using qPCR, or fluorescence method such as FitAmp General DNA Quantification (Cat# P-1020, EpigenTek), Qubit or Picogreen assays. (4) Each indexed library can be combined in equal amounts to form multiplexed libraries for sequencing.*

Store the prepared library at -20ºC until ready to use for sequencing.

TROUBLESHOOTING

Problem	Possible Cause	Suggestion
Little or no library cDNA generated from sample.	Insufficient amount of qualified RNA or m^6A-containing RNA	Use higher amount of RNA
	Poor enrichment with antibody	Check if the immunocapture condition is correct
	Inappropriate RNA fragmenting condition.	**Cleavage Enzyme Mix** (**CEM**) may be degraded due to improper temperature storage. Ensure the proper storage condition for this component **Cleavage Enzyme Mix (CEM)** reaction time is too short or too long. Under this condition, the cleavage should be optimized to allow RNA fragment size to be between 30-200 bps.
	Incorrect temperature and/or insufficient time during RNA release.	Ensure the 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 library DNA 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 this component is sufficient for 4 extra washes for each sample.
Unexpected peak size of Agilent® Bioanalyzer® trace: presence of <150 bp adaptor dimmers or presence of larger fragments than expected	Improper ratio of **MQ Binding Beads** to DNA volume in size selection.	Check if the correct volume of **MQ Binding Beads** is added to DNA solution accordingly. Proper ratios should remove the fragments with unexpected peak sizes.
	Over-amplification of library.	PCR artifacts from over-amplification of the library may cause the fragment population to shift higher than expected. Make sure to use proper PCR cycles to avoid this problem.

RELATED PRODUCTS

RNA Methylation Quantification

P-9003	Methylamp™ RNA Bisulfite Conversion Kit
P-9005	EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric)
P-9008	EpiQuik™ m6A 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-9018	EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) 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 Tissue (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 Sensitive DNA Library Prep Kit (Illumina)

NGS Barcode

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

For ChIP-grade antibodies, search "chip-grade" at www.epigentek.com

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