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ADVANCEMENTS IN RNA MODIFICATION TECHNOLOGIES

A review of some of the most common methods for studying RNA modifications, such as m6A, and notable advancements in epitranscriptomic technologies over the past decade.

Advancements in RNA Modification Technologies

There is currently an upsurge of interest in epitranscriptomics and modified RNA, especially in light of the recent pandemic concern surrounding SARS-CoV-2, the RNA virus behind the COVID-19 outbreak. Post-transcriptional modifications are known to play a part in the life cycles of certain viruses like the human coronavirus. Adenosine methylation in particular, such as m6A, has been reported to affect the viability of specific RNA viruses by modulating viral cap structures, viral replication, innate sensing pathways, and the innate immune response [1]. Interestingly, m6A exhibits both pro- and anti-viral activities, depending on the virus species and host cell type [2]. This makes the viral epitranscriptome an attractive target for remedial intervention.

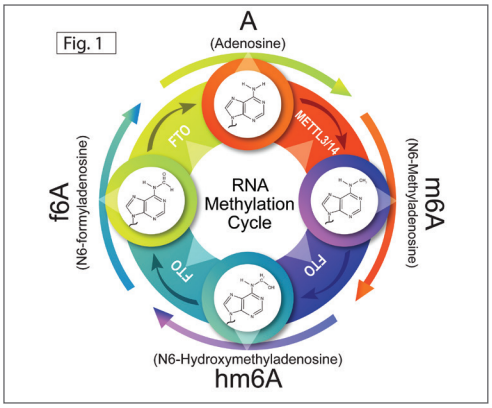


Fig. 1. The m6A RNA cycle. *The formation of m6A RNA is mediated by a multi-protein methyltransferase complex composed, in part, of METTL enzymes. A methyl group is chemically added at the nitrogen-6 position of A residues by the METTL3/14 heterodimer to form m6A. Through FTO-mediated oxidative demethylation, m6A is converted in a step-wise manner to hm6A and subsequently f6A before finally reverting back to A.*

Often referred to as "the fifth RNA base", N6-methyladenosine, or m6A, is the most common and abundant eukaryotic RNA modification, accounting for over 80% of all RNA methylation. It can be found mainly in mRNA, but is also observed in non-coding species like tRNA, rRNA, and miRNA. Through interactions with various binding proteins called "readers", m6A affects virtually every facet of ribonucleic acid biology: structure, splicing, localization, translation, stability, and turnover [3]. Aside from this central role in RNA metabolism, m6A is a factor in other physiological processes such as cell differentiation, immunity, inflammation, and the circadian clock [4]. Abnormal m6A methylation has been implicated in diverse pathologies: diabetes, obesity, neurodegeneration, and cancer, to name a few. The formation of m6A RNA appears to be a co-transcriptional event occurring early on in the RNA lifecycle and is mediated by a multi-protein methyltransferase complex (Fig. 1). Members of the coronavirus and non-CoV species encode their own methyltransferases for self-methylating adenosine residues and promoting immune evasion.

This synopsis describes some of the most common methods of studying RNA modifications and highlights notable advancements in epitranscriptomic technologies (Fig. 2).

ANTIBODY-BASED METHODS

Dot blot: In comparison with other methods, dot blot analysis of global modified RNA levels is a simpler, faster, and more cost-effective approach. The technique involves the blotting of RNA samples directly onto a membrane substrate, each sample at a single spot in the form of a circular dot. Subsequent to air drying and blocking of non-specific binding sites, the membrane is incubated with a specific antibody (e.g., anti-5mC or -m6A) for modified nucleoside detection of spotted RNA.

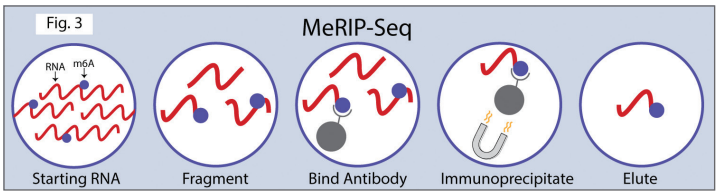
Immuno-northern blotting: INB, or immuno-northern blotting, is another immunoblotting method for antibody-based detection of modified RNA [5]. In INB, sample RNA is first size-separated via gel electrophoresis prior to membrane transfer and antibody incubation. This initial size separation step of the INB protocol enables the identification of modified nucleosides from different RNA types (e.g., tRNA, rRNA) within total RNA specimens, which is not possible with dot blot analysis.

ELISA:

Global RNA methylation quantification: RNA methylation ELISAs are a simple and convenient way to rapidly assess global levels of a specific methylated RNA modification before pursuing more in-depth and costly applications such as qPCR and NGS. While analogous in terms of assay workflow (e.g., sample binding to assay wells, target capture by primary antibody, signal detection with enzyme-conjugated secondary antibody), compared with conventional ELISAs, RNA methylation ELISAs are extremely sensitive with much lower detection limits, allowing for the assessment of a wider range of sample types. Whether the target is 5mC or m6A, RNA methylation ELISAs provide a high-throughput, cost-effective means of analyzing a variety of modified RNA forms. As pioneers in the R&D of epigenetics-based research tools, EpiGentek has leveraged its proprietary MethylFlash and EpiQuik technologies to develop rapid, accurate, and highly cited quantitation immunoassays for global RNA methylation analysis. These assays have been specially designed to use fully intact input RNA as starting material, eliminating the need for additional sample processing (e.g., denaturation, fragmentation) required by similar assays.

m6A methyltransferase activity: The recent discoveries of m6A methylase "writers" and their associated demethylase "erasers" in mammals uncovered the reversibility of the m6A modification, exposing potential therapeutic targets for m6A dysregulation-related diseases. EpiGentek's ELISA-based Epigenase assays provide rapid measures of m6A methylase and demethylase activity levels from cell/tissue nuclear extracts or purified enzymes in a high-throughput format. A unique m6A substrate is stably coated on the assay wells. Bioactive enzymes from input samples will transfer methyl groups to (methylases), or remove them from (demethylases), the bound substrate. Substrate m6A methylation is subsequently detected by a high-affinity antibody specific for this modification. Quantitative measurements of methylase/demethylase activity and inhibition (for enzyme inhibitor screening) can be quickly obtained within only a few hours.

Fig. 3. MeRIP-seq assay overview.



MeRIP-seq: The development of methylated RNA immunoprecipitation sequencing (MeRIP-seq) was a landmark in the field of epitranscriptomics as it was the first method to detect m6A on a transcriptome-wide level [6]. MeRIP-seq couples m6A RNA immunoprecipitation with NGS, allowing for high-throughput localization of modified sites from enriched m6A-containing RNA fragments that have been precipitated by a specific antibody, reverse-transcribed, and sequenced (Fig. 3). The fragment sizes generated during the random fragmentation step preceding immunoprecipitation limits the precise mapping of the m6A site to within a ~200 nt stretch. The choice of antibody allows MeRIP-seq to be adapted toward the study of other modified RNA types (e.g., 5hmC RNA, [7]).

miCLIP: m6A individual-nucleotide resolution crosslinking and immunoprecipitation (miCLIP) was designed to address the disadvantages associated with MeRIP-seq approaches regarding the mapping of m6A RNA sites at individual-nucleotide resolution [8]. The key feature of this method is the UV-crosslinking of immunoprecipitated RNA fragments to the capture antibody. Antibody remnants at the crosslinking site on the RNA after Proteinase K treatment induce signature mutations (truncations and C→T transitions) during cDNA synthesis that can be identified by sequencing and used to more precisely map the specific m6A location. These antibody-induced mutational signatures have also been successfully applied to the mapping of m6Am RNA modifications.

PA-m6A-seq: Photo-crosslinking-assisted m6A sequencing (PA-m6A-seq) is an alternative UV-based strategy that was fashioned for high-resolution (~23 nt) transcriptome-wide m6A mapping [9]. This method employs a photoreactive ribonucleoside crosslinker to induce a signature mutation for localizing m6A, akin to miCLIP. The uridine analogue 4-thiouridine (4SU) is incorporated into sample RNA. Full-length, unfragmented, 4SU-labeled RNA molecules are then immunoprecipitated with an anti-m6A antibody, and UV irradiation is applied to establish covalent crosslinks between m6A-bound antibody and neighboring 4SU. Crosslinked RNA is digested with RNase T1 to yield ~30 nt-long fragments that are further processed for library preparation and sequencing, whereby crosslink-generated T→C transitions adjacent to the m6A sites can be identified.

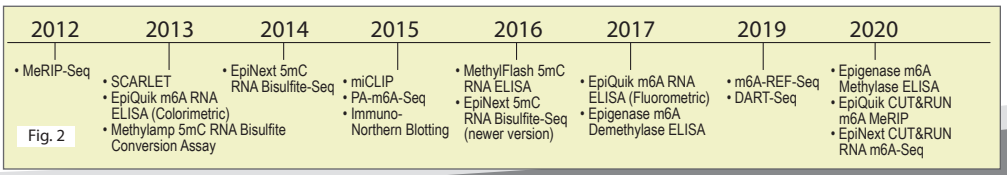
CUT&RUN m6A MeRIP: Established methods used for epitranscriptome-wide m6A mapping like MeRIP-seq, PA-m6A-seq, and miCLIP have either been widely used but are unable to achieve high resolution in m6A profiling, or improve the profiling resolution but suffer from poor reproducibility and a complicated process. In particular, they are time-consuming (> 2 days) and costly. To address these issues, EpiGentek has developed a new method: cleavage under target and recover using nuclease for m6A enrichment (CUT&RUN m6A MeRIP). This innovative approach combines the advantages of MeRIP-seq and miCLIP with EpiGentek's proprietary EpiQuik technology for higher enrichment, lower input, reduced background, and a faster, more streamlined procedure. CUT&RUN m6A MeRIP uses a state-of-the-art RNA cleavage enzyme mix to simultaneously fragment immunocaptured 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 consequently generated only bound with anti-m6A antibody. True target m6A-enriched regions can therefore be reliably identified, and high-resolution mapping achieved.

ANTIBODY-FREE METHODS

HPLC/MS: Considered "the gold standard" for quantitative analysis of modified nucleic acid bases, high performance liquid chromatography, together with mass spectrometry (HPLC/MS), has been routinely applied in the quantification of RNA methylation. A typical HPCL/MS protocol initially involves nuclease P1 and alkaline phosphatase digestion of isolated RNA. Following digested RNA purification, ribonucleosides are separated by reverse phase HPLC on a C18 column. Ribonucleoside mass chromatograms are then acquired by tandem mass spectrometry, and the target modification is quantified based on a standard curve of pure modified ribonucleoside standards. Although highly quantitative and reproducible, HPLC/MS requires expensive instrumentation, lengthy and time-consuming protocols, specialized technical expertise, and trained personnel.

Bisulfite conversion: Treating RNA with bisulfite, followed

Fig. 2. *Timeline for select technological innovations in epitranscriptomics over the past decade.*



by RT-PCR amplification, cloning, and sequencing, yields reliable information about RNA cytosine methylation states. Bisulfite conversion is a chemical reaction whereby unmethylated cytosines are deaminated to uracil while 5mC is left intact. As a consequence, methylated and unmethylated RNA cytosine residues are differentiated from one another. The traditional conversion method requires a lengthy protocol time that leads to heavy RNA degradation, high inappropriate 5mC deamination, and low cytosine conversion rate. Efforts by EpiGentek to condense the entire bisulfite process to just a few hours have significantly improved cytosine conversion efficiency and effectively prevent bisulfite-treated RNA degradation.

SCARLET: Site-specific cleavage and radioactive-labeling followed by ligation-assisted extraction and thin-layer chromatography (SCARLET) was originally devised as a way to identify m6A RNA sites at single-nucleotide resolution, but can be customized for different RNA modifications [10]. Target RNA is firstly annealed to a complementary 2'-OMe/2'-H chimeric oligonucleotide that guides the RNase H-mediated cleavage of bound RNA 5' to the site of interest. The cleaved site, along with all other RNA 5' ends, are subsequently radiolabeled with 32P. The 32P-labeled target fragment is then selectively ligated to a 116-mer ssDNA sequence via a splint oligonucleotide that hybridizes to both on opposite ends (3' end of the ssDNA-116 and 5' end of the RNA target) and serves as a bridge between the two during ligation. After RNase T1/A treatment, the splint-ligated product, which is now in the form of DNA-32P-(A/m6A)p and DNA-32P-(A/m6A)Cp (in the case of m6A candidates), can be isolated with denaturing PAGE based on its predicted 117/118-mer migration pattern. Finally, m6A and unmethylated adenosine mononucleotides generated by nuclease P1 digestion can be separated using TLC and phosphorimaged by their radiolabels to determine RNA modification status.

m6A-REF-seq: m6A-sensitive RNA-Endoribonuclease-Facilitated sequencing, or m6A-REF-seq, is an antibody-independent method recently engineered to identify m6A sites transcriptome-wide in single-base resolution [11]. m6A-REF-seq utilizes the E. coli toxin and RNA endoribonuclease MazF to distinguish between m6A and unmethylated adenosine. The enzyme specifically targets unmethylated ACA motifs for cleavage, while methylated (m6A)CA motifs are left intact [12]. First, RNA samples are subjected to MazF-mediated fragmentation. The resulting RNA fragments then undergo end repair, purification, adaptor ligation, PCR amplification, and NGS. Intact ACA motif sequences are indicative of m6A methylation. However, as RNA secondary structure interferes with MazF activity, the utility of this enzyme is limited to single-stranded regions.

DART-seq: Another antibody-free method for m6A detection is deamination adjacent to RNA modification sites (DART-seq), a novel approach that selectively deaminates cytosines contiguous to m6A residues [13]. The selective deamination is mediated by a unique APOBEC1—m6A-binding YTH domain fusion protein, which converts m6A-adjacent cytosine bases to uracils while leaving unmethylated A-C motifs intact. Methylated and unmethylated sites can then be discerned by way of standard RNA-seq. This highly specific technique allows the use of very low amounts of input material to obtain m6A maps. Unlike m6A-REF-seq and SCARLET, which can only be used to identify a subset of m6A sites, DART-seq enables simultaneous single-nucleotide resolution m6A detection and quantification of the entire transcriptome.

Advanced antibody-free m6A detection strategies like SCARLET, m6A-REF-seq, and DART-seq have been useful for measuring the amount of m6A on the level of individual sites. However, intricate protocols, inordinate expenses, and the ongoing need for a reliable method for global modified RNA quantification have limited their ubiquitous utility. Although antibody-based methods typically require higher starting RNA amounts, their convenience, low-cost, speed, and maturity make them the dominant means for epitranscriptomic research, with ELISA and MeRIP still being the most popular approaches. As investigators press ahead to work out the kinks, better-quality antibodies with improved target specificity and minimal off-target binding will be essential for more efficient RNA modification assessment.

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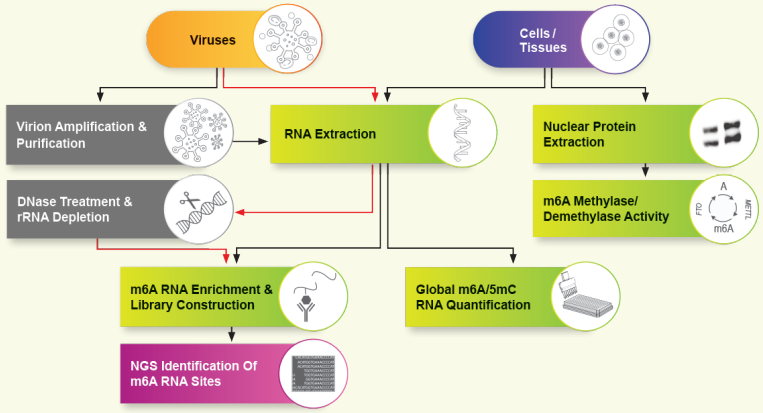
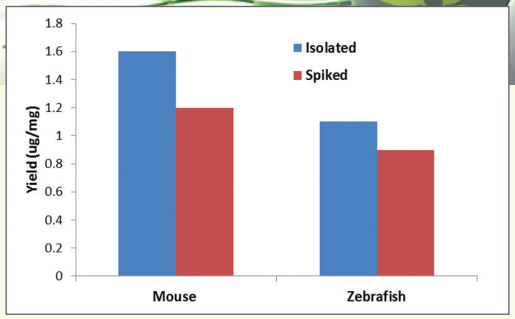
End-to-End Workflow for Viral & Host RNA Modification Analysis

EpiGentek provides a complete set of workflow solutions for viral RNA modification research in the form of kits and information. To view the interactive chart (shown below) that explains how each part plays into an experiment where RNA modifications of either cells, tissues, or viruses can be studied, please visit: **www.epigentek.com/workflow-rna**



Rapid RNA Isolation Kits



Total RNA was extracted from mouse liver tissue (~6 mg) and Zebrafish tissue (10 mg) using the EpiQuik™ Total RNA Isolation Fast Kit (Cat. #P-9105).

Quickly and easily isolate high-quality, intact RNA from cells or tissue for use in a variety of routine applications.

- High-quality total RNA in 30 minutes
- Ready-to-use in any downstream application
- Consistent RNA yield from a small input amount

Cat. #	Product Name
P-9105	EpiQuik Total RNA Isolation Fast Kit
P-9106	EpiQuik Magbeads Quick RNA Isolation Kit

*Also available: Viral RNA extraction kits. (See Cat. #s P-9107, P-9108, P-9109)

See Our Interactive Workflow for Viral RNA Studies:
www.epigentek.com/workflow-rna

RNA methylation is a reversible post-translational modification to RNA that epigenetically impacts numerous biological processes. It occurs in different RNAs, including tRNA, rRNA, mRNA, tmRNA, snRNA, snoRNA, miRNA, and viral RNA. Different catalytic strategies are employed for RNA methylation by a variety of RNA methyltransferases.

N6-methyladenosine (m6A or 6mA) is the most common and abundant methylation modification in RNA molecules present in eukaryotes and accounts for more than 80% of all RNA methylation. It may be referred to as the “fifth RNA base” and has broad roles in regulating embryonic development and cell fates. In particular, this RNA modification is thought to be important for mRNA regulation due to the enrichment of m6A at 3’UTRs. Abnormal m6A methylation has been linked to cancers, including leukemia, breast cancer, and prostate cancer.

Directly Measure m6A in RNA Samples

Expand upon your understanding of RNA methylation with the help of EpiGentek’s complete suite of m6A RNA methylation products. Directly quantify the "fifth RNA base" from total RNA isolated from a variety of samples and a wide range of species, including mammals, plants, fungi, bacteria, and viruses.

ELISA-LIKE KITS TO STUDY m6A RNA METHYLATION:

EpiQuik m6A RNA Methylation Quantification Kits
Directly quantify m6A in RNA using total RNA isolated from any species

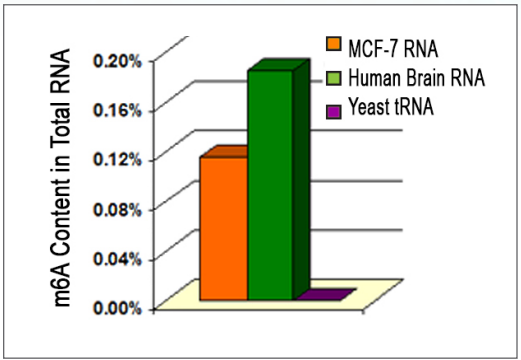
MethylFlash Urine N6-methyladenosine (m6A) Quantification Kit
Directly quantify m6A in RNA from urine samples

Epigenase m6A Demethylase Activity/Inhibition Assay Kit
Measure activity or inhibition levels of total m6A demethylases from nuclear extracts or purified m6A demethylases

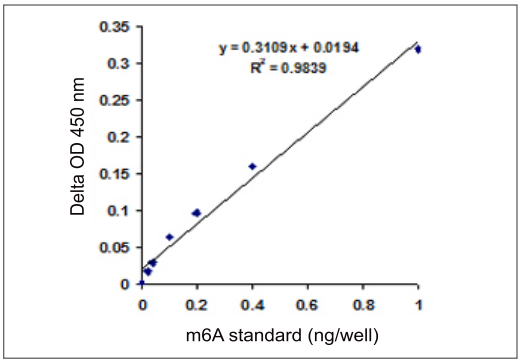
Epigenase m6A Methylase Activity/Inhibition Assay Kit
Measure the activity/inhibition of total m6A methylases using nuclear extracts or purified enzymes from a broad range of species

Cat. #	Product Name
P-9005*	EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric)
P-9008	EpiQuik™ m6A RNA Methylation Quantification Kit (Fluorometric)
P-9015	MethylFlash™ Urine N6-methyladenosine (m6A) Quantification Kit (Colorimetric)
P-9013	Epigenase™ m6A Demethylase Activity/Inhibition Assay Kit (Colorimetric)
P-9019	Epigenase™ m6A Methylase Activity/Inhibition Assay Kit (Colorimetric)

See Our RNA Methylation Kits:
www.epigentek.com/rna-methylation-kits



▲ Quantification of m6A RNA methylation in different samples. 200 ng of RNA isolated from different tissues or cells were added into the assay wells and the m6A contained in RNA was measured using the **EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric) (Cat. #P-9005)**.



▲ m6A standard control was added into the assay wells at different concentrations and then measured with the **EpiQuik™ m6A RNA Methylation Quantification Kit (Colorimetric) (Cat. #P-9005)**.

***Highly Rated & Highly Cited** (100+ Publications)

★★★★★
*“The protocol was easy to follow, and the results were consistent.” by I******@cabnr.unr.edu*

★★★★★
*“This is our go-to kit. The instructions are easy to follow, the results are reproducible.” by c*******@gmail.com*

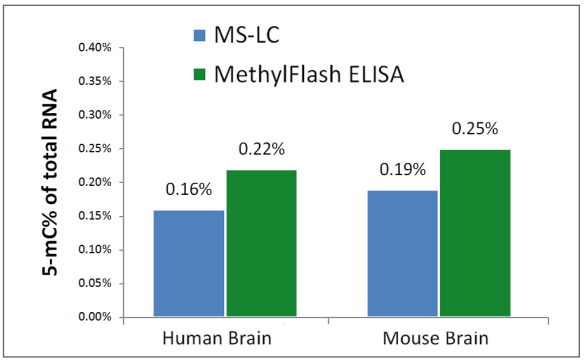
Quantify 5-mC in RNA Samples

The occurrence of 5-methylcytosine (5-mC) in RNA is well documented in various RNA molecules, including tRNAs, rRNAs, mRNAs, and ncRNAs. RNA cytosine methylation affects many biological processes, like RNA stability and mRNA translation. Loss of 5-mC in vault RNAs can cause aberrant processing into Argonaute-associated small RNA fragments that can function as microRNAs.

MethylFlash 5-mC RNA Methylation ELISA Easy Kit
Global 5-mC RNA methylation quantification of total RNA isolated from any species

Methylamp RNA Bisulfite Conversion Kit
Robust, chemical-based bisulfite treatment of RNA for methylated cytosine analysis

EpiNext 5-mC RNA Bisulfite-Seq Easy Kit (Illumina)
Streamlined RNA bisulfite conversion and NGS library preparation for downstream sequencing



▲ Accurate quantification of 5-mC content of various RNA samples from different species using the **MethylFlash™ 5-mC RNA Methylation ELISA Easy Kit (Fluorometric) (Cat. #P-9009)**. The results closely correlate with those obtained by MS-LC.

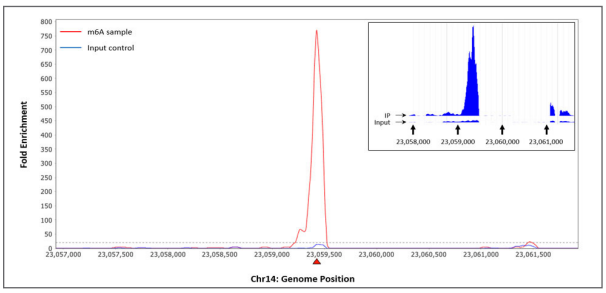
Cat. #	Product Name
P-9009	MethylFlash™ 5-mC RNA Methylation ELISA Easy Kit
P-9003	Methylamp™ RNA Bisulfite Conversion Kit
P-9007	EpiNext™ 5-mC RNA Bisulfite-Seq Easy Kit (Illumina)

CUT&RUN m6A Enrichment

Mapping m6A across the transcriptome is essential for investigating its biological functions. MeRIP and MeRIP-seq are useful in studying the regulatory roles of m6A, but have limitations and are costly. To improve upon these methods, EpiGentek developed **CUT&RUN RNA m6A enrichment** (cleavage under target and recover using nuclease for RNA m6A enrichment). Utilizing a unique nucleic acid cleavage enzyme mix, this approach isolates only RNA segments at the antibody binding locations, allowing for high-resolution mapping even when RNA input is small. Plus, the simultaneous capture and cleavage under target method streamlines the procedural steps, going from RNA to library cDNA in just hours. Kits using this method include:

EpiQuik CUT&RUN m6A RNA Enrichment (MeRIP) Kit
MeRIP based on CUT&RUN for increased specificity and signals

EpiNext CUT&RUN RNA m6A-Seq Kit
All-in-one MeRIP & NGS library preparation based on CUT&RUN



◀ The m6A peak distribution within the human ACIN1 transcript from samples processed with the EpiQuik CUT&RUN m6A RNA Enrichment (MeRIP) Kit (Cat. #P-9018) correlates well with expected regions. This example peak demonstrates a 760 fold enrichment with a sample.

Cat. #	Product Name
P-9018	EpiQuik™ CUT&RUN m6A RNA Enrichment (MeRIP) Kit
P-9016	EpiNext™ CUT&RUN RNA m6A-Seq Kit

Measure 5mC/5hmC in 2 Hours

Best Seller

Quantify global DNA methylation or hydroxymethylation status by specifically measuring levels of 5-mC or 5-hmC in a simplified, “one-step” ELISA-like format.

- Finish in 2 hours
- Reduced variation between replicates
- Inherently low background noise
- Detects as low as 0.05% methylated or 0.01% hydroxymethylated DNA from 100 ng of input.
- No cross-reactivity with other cytosines
- Suitable for any species
- Highly Comparable with HPLC-MS analysis
- Flexible Strip-well microplate format

Cat. #	Product Name
P-1030	MethylFlash™ Global DNA 5-mC ELISA Easy Kit
P-1032	MethylFlash™ Global DNA 5-hmC ELISA Easy Kit

See Our MethylFlash Easy Kits: www.epigentek.com/methylflash-kits

CUT&RUN / CUT&Tag

Advanced Methods for Chromatin Mapping

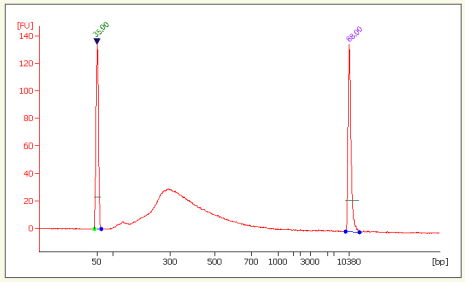
CUT&RUN and CUT&Tag are prominent innovations in the study of protein/DNA interactions, designed to address the limitations of the popular ChIP-seq technique, namely: cross-linking artifacts that can induce epitope masking and false-positive signals; random shearing of extracted chromatin resulting in immunoprecipitated DNA fragments of varying length (typically several hundred base pairs long) and thus poor resolution of interaction sites; and the large amount of starting material required to produce strong enough signals over background noise.

By simultaneously tethering protein-bound primary antibody and enzymatically cleaving chromatin near the protein/DNA binding region, in situ within live cells or isolated nuclei, the CUT&RUN and CUT&Tag methods eliminate interference from cross-linking and yield shorter-length DNA fragments, allowing for reduced background signals. CUT&Tag has the added feature of combining cleavage and library preparation into a single step via NGS adapter-loaded fusion protein.

Despite these advantages, enzymatic over-digestion of DNA, high costs, and lengthy protocol times are still problematic for CUT&RUN and CUT&Tag. Accordingly, EpiGentek has developed two new assays, the EpiNext™ CUT&RUN Fast Kit and the EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit. These novel approaches integrate the benefits of CUT&RUN and CUT&Tag with the fastest procedures in convenient all-in-one kits to rapidly, cost-effectively, and reliably identify true target protein-enriched regions. High-resolution mapping with minimal background is achievable from very low sample input amounts. Library DNA can be generated in just a few hours starting from as little as 500 cells or 100 ng of chromatin extract.

EpiNext CUT&RUN Fast Kit
Enrich DNA sequences from target protein/DNA complexes in less than 3 hours. The enriched product is suitable for downstream library construction and sequencing

EpiNext cTIP (CUT&Tag In-Place)-Sequencing Kit
Couple target enrichment with in-place tagging for NGS library preparation in under 5 hours



Size distribution of library fragments using the EpiNext cTIP (CUT&Tag In-Place)-Sequencing Kit (Cat. # P-2032): Histone/DNA complex was captured from 500 ng of chromatin isolated from Hela cells by the control antibody (H3K9me3) and used for DNA library preparation. Peak at 295 bps reflects the insert size of mononucleosome (around 150 bps).

Cat. #	Product Name
P-2028	EpiNext™ CUT&RUN Fast Kit
P-2032	EpiNext™ cTIP (CUT&Tag In-Place)-Sequencing Kit

See Our Chromatin Immunoprecipitation Kits:
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Microplate-Based ChIP Kits

EpiGentek debuted the high throughput microplate-based ChIP format in 2005, enabling rapid and low-cost immunoprecipitation of chromatin samples for epigenetic researchers. Each kit contains everything you need to reliably immunoprecipitate chromatin in a fast, efficient manner.

Cat. #	Product Name
P-2027	ChromaFlash™ High-Sensitivity ChIP Kit
P-2014	EpiQuik™ Plant ChIP Kit
P-2030	EpiNext™ ChIP-Seq High-Sensitivity Kit (Illumina)

Histone H3 & H4 Modifications

Histone H3 Modification Multiplex Kit
Simultaneously screen and measure 21 different histone H3 modification patterns

Modifications: H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me1, H3K27me2, H3K27me3, H3K36me1, H3K36me2, H3K36me3, H3K79me1, H3K79me2, H3K79me3, H3K9ac, H3K14ac, H3K18ac, H3K56ac, H3ser10P, H3ser28P, Total H3

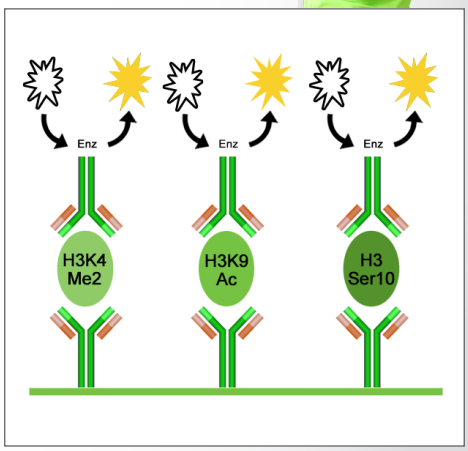
Histone H4 Modification Multiplex Kit
Simultaneously screen and measure 10 different histone H4 modification patterns

Modifications: H4K5ac, H4K8ac, H4K12ac, H4K16ac, H4R3me2a, H4R3me2s, H4K20me1, H4K20me2, H4K20me3, H4ser1, Total H4

Circulating Modified Histone H3 Multiplex Kit
Simultaneously screen and measure 22 circulating histone H3 modification patterns

Modifications: H3K4me1, H3K4me2, H3K4me3, H3K9me1, H3K9me2, H3K9me3, H3K27me1, H3K27me2, H3K27me3, H3K36me1, H3K36me2, H3K36me3, H3K79me1, H3K79me2, H3K79me3, H3K9ac, H3K14ac, H3K18ac, H3K27ac, H3K56ac, H3ser10P, H3cit, Total H3

Cat. #	Product Name
P-3100	EpiQuik™ Histone H3 Modification Multiplex Assay Kit
P-3102	EpiQuik™ Histone H4 Modification Multiplex Assay Kit
P-3106	EpiQuik™ Circulating Modified Histone H3 Multiplex Assay Kit



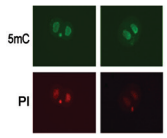
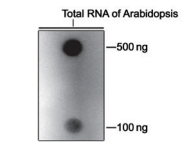
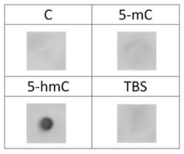
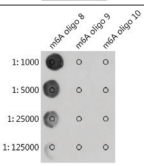
▲ Working principle of the EpiQuik™ Histone H3 Modification Multiplex Assay Kit. (Cat. #P-3100)

See Our Histone Modification Kits:
www.epigentek.com/histone-mod-kits

Validated Epigenetic Antibodies

DNA & RNA Modification Antibodies

High-quality, validated DNA and RNA modification antibodies targeting the most common and widely-studied marks, including 5-mC, 5-hmC, and m6A -- the same antibodies that are the driving force behind our successful MethylFlash technology.

	5-mC mAb [33D3] (#A-1014) DB, ELISA, IF, IHC, IP, MeDIP		m6A pAb (#A-1801) DB, ELISA, IF, IP, MeRIP
	5-hmC mAb [HMC/4D9] (#A-1018) DB, ELISA, hMeDIP, IF, IHC		m6A mAb [2H6] (#A-1802) DB, ELISA, IF, MeRIP, Nucleotide Array

Histone Modification Antibodies

First-rate, validated histone antibodies targeting various chemical modifications, including acetylation, methylation, and phosphorylation -- the same antibodies we use in our successful and highly-cited research assay kits.

H3K9ac (#A-4022) ChIP, ChIP-seq, IF, IHC, IP, WB	H3K9me2 (#A-4035) ChIP, ChIP-seq, IF, IHC, IP, WB	H4K5ac (#A-4027) ELISA, IF, IHC, WB	H4K8ac (#A-4028) ChIP, ELISA, IF, IHC, WB
H3K18ac (#A-4024) ChIP, ChIP-seq, IF, IHC, IP, WB	H3K27me3 (#A-4039) ChIP, ChIP-seq, IF, IHC, IP, WB	H4K12ac (#A-4029) ELISA, IF, IHC, WB	H4K16ac (#A68398) ELISA, IF, IHC, WB
H3K36me3 (#A-4042) ChIP, ChIP-seq, IF, IHC, IP, WB	H3R8me2s (#A-3706) ChIP, ChIP-seq, IF, IHC, IP, WB	H4K20me3 (#A-4048) ChIP, ChIP-seq, IF, IHC, IP, WB	H4R3me2s (#A-3718) IF, IHC, WB

See All Our Antibodies:
www.epigentek.com/epi-antibodies

100% Satisfaction Guarantee
All EpiGentek products are covered by our 6-month money back guarantee

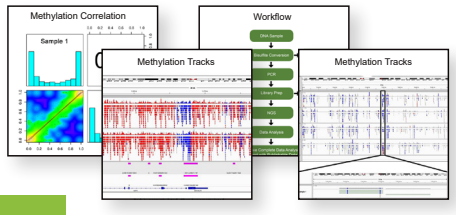
RRBS/WGBS Services

Explore A Wide Range of Species

EpiGentek offers a suite of epigenetic services, including RRBS, WGBS, and targeted bisulfite-seq for genome-wide, base resolution DNA methylation analysis.

- *Coverage of All CpGs*
- *>99.9% Conversion & Maximum Recovery*
- *Minimal Selection Bias & Low Input Requirements*
- *High Mapping & Low Error Rates*
- *Illumina-Based NGS Technology*

▼ Receive high quality data ready for publication.



See Our Epigenetic Services:
www.epigentek.com/epi-services

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THE DECODER

A NEWSLETTER FOR EPIGENETIC RESEARCHERS

The Decoder • 2021 • Issue 5

RNA Methylation Analysis Tools

2-Hour Global DNA Methylation

Screen for Histone Modifications

Advantages of CUT&RUN / CUT&Tag

Validated Epigenetic Antibodies

Epigenetic Services: RRBS & WGBS