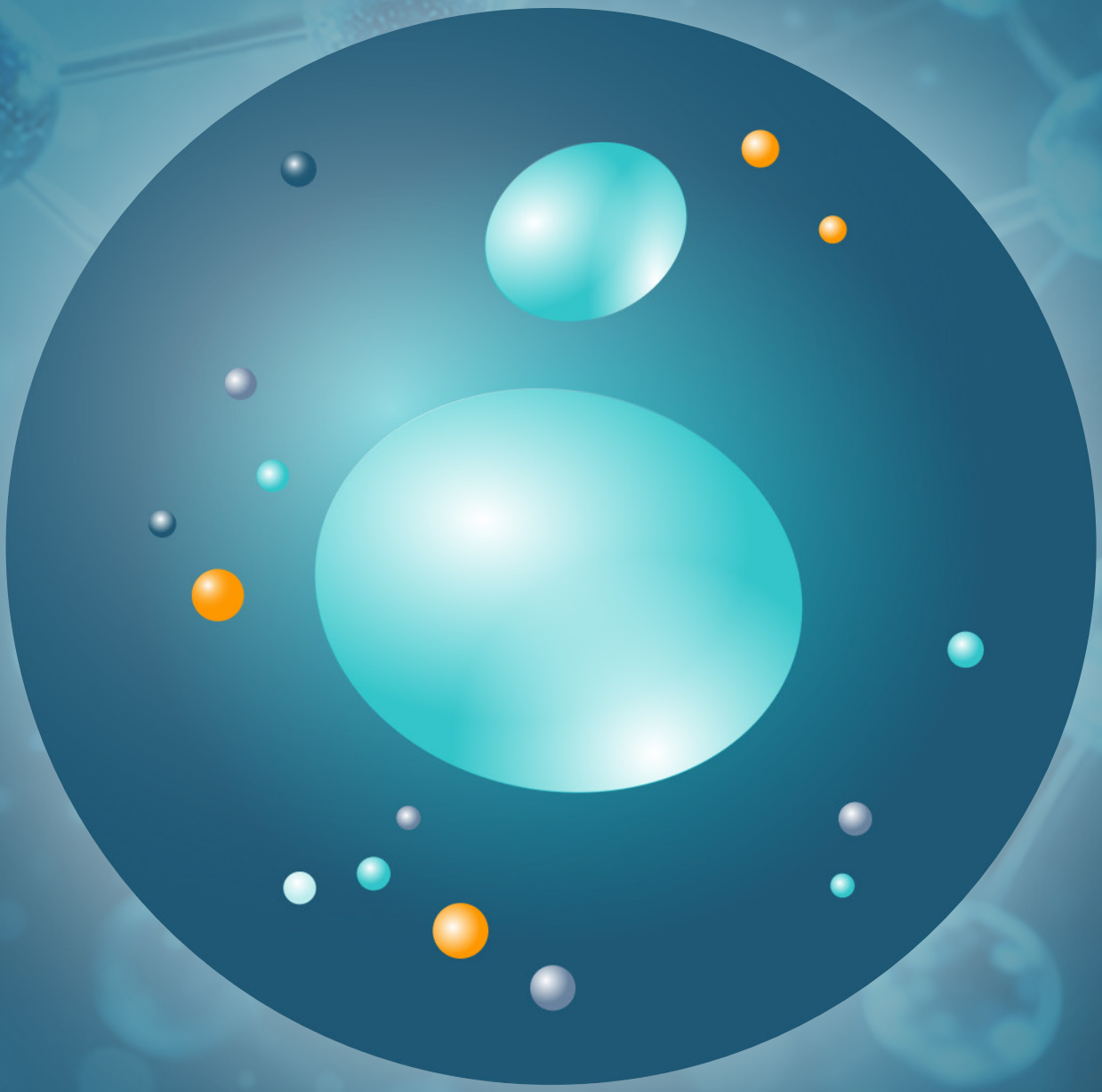
EpiFinder™ cNUC

Unlocking the Epigenetic Dimension of Liquid Biopsy



Epigenica

Unlocking the Epigenetic Dimension of Liquid Biopsy

Most cell-free DNA in blood exists in the form of circulating nucleosomes (cNUCs). A substantial fraction of these cNUCs originates from different tissues in the body and retains epigenetic marks that reflect the gene expression programs of their cells of origin. A single nucleosome consists of about 160 base pairs of DNA sequence wrapped around a core of histone proteins.

While liquid biopsies are widely used for genetic analysis, the epigenetic information carried by cNUCs has remained largely unexplored - until now.

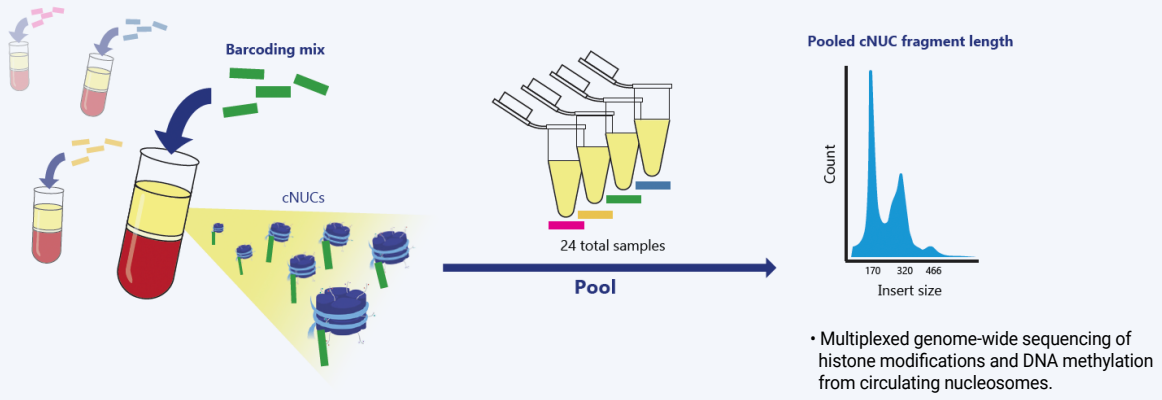
> EpiFinder™ cNUC is the first solution on the market enabling high-throughput, genome-wide, multiplexed epigenetic profiling directly from plasma or serum, opening a new dimension for liquid biopsy research.

Why Epigenetic Analysis in Liquid Biopsy Matters

Genetic alterations provide only part of the biological picture. Epigenetic regulation, including histone modifications and DNA methylation, governs gene expression, cell identity, and disease state. Accessing this regulatory information from blood enables:

- **Insight into tissue-of-origin and transcriptional activity**
- **Detection of regulatory changes beyond DNA sequence variation**
- **Expanded opportunities for biomarker discovery and translational research**

EpiFinder™ cNUC makes this information accessible directly from standard plasma or serum samples.



- Multiplexed genome-wide sequencing of histone modifications and DNA methylation from circulating nucleosomes.

From Epigenetic Insight to Biological Impact in Liquid Biopsy

EpiFinder™ cNUC reveals a previously inaccessible regulatory layer in liquid biopsy by extracting rich epigenetic information from circulating nucleosomes. This enables a broad spectrum of discovery and translational applications, including:

DECODING REGULATORY ACTIVITY
across health and disease, including cancer, inflammation, and aging-related processes

BIOMARKER DISCOVERY AND VALIDATION
powered by high resolution chromatin signatures

TISSUE OF ORIGIN AND TRANSCRIPTIONAL STATE PROFILING
through nucleosome associated histone marks

LONGITUDINAL MONITORING
of disease progression, therapeutic response, and minimal residual disease

Product Overview

The EpiFinder™ cNUC Kit enables quantitative, multiplexed epigenetic profiling directly from liquid biopsy samples, without the need for prior DNA purification.

The kit supports parallel analysis of histone post-translational modifications (hPTMs) and DNA methylation from plasma or serum. The kit includes all required reagents and enables, in just a single run, profiling of:

- **Up to five histone PTMs plus DNA methylation, or**
- **Up to six histone PTMs,**

across 24 liquid biopsy samples.

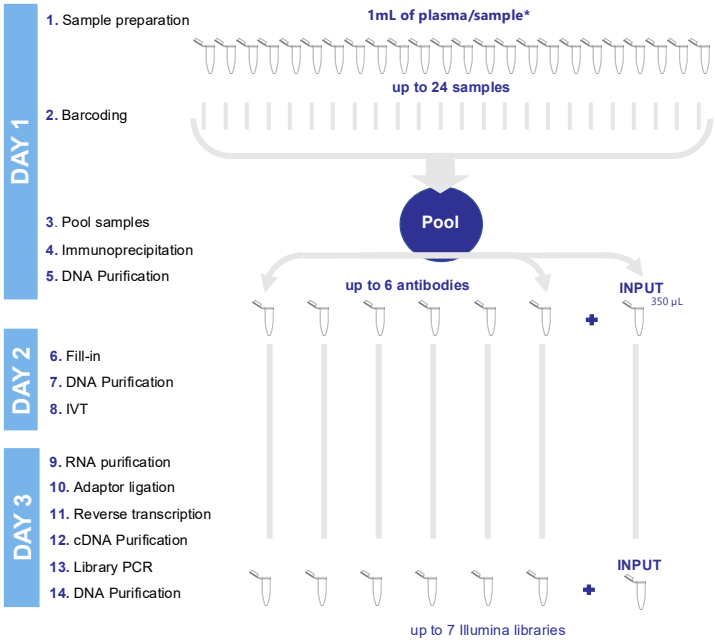
The workflow generates seven sequencing libraries, including an input control. All libraries are barcoded, pooled, and sequenced together on a single flow cell, producing up to 168 genome-wide coverage tracks per run. The input library is used for normalization and can additionally support the detection of large-scale copy number variations.

Streamlined Multiplexed Workflow

The EpiFinder™ cNUC workflow is completed over three days with approximately 14 hours of hands-on time.

Samples are barcoded and pooled, enabling parallel processing of up to 24 plasma or serum samples in a single run. Multiplexed immunoprecipitation is performed using up to six validated antibodies, together with an input control, followed by library preparation to generate up to seven Illumina-compatible sequencing libraries per sample.

The resulting libraries are barcoded and sequenced on an Illumina flow cell. The streamlined workflow is guided by an optimized protocol and is accessible to researchers without prior ChIP-seq experience.



Seamless Data Processing with an Open-Source Pipeline

EpiFinder™ cNUC is supported by a fully open-source data processing pipeline, enabling transparent, reproducible, and scalable analysis of epigenomic liquid biopsy data.

From raw sequencing output to analysis-ready data, the pipeline provides a streamlined, end-to-end workflow, including:

- **Raw data quality control**
- **Sample demultiplexing**
- **Genome alignment**
- **Signal track generation (normalized coverage profile per marker and sample)**

The resulting datasets consist of genome-wide signal coverage tracks, ready for immediate use in downstream analyses such as peak calling, differential analysis, integrative multi-omics, or custom analytical workflows.

The open-source design ensures full transparency, flexibility, and compatibility with existing bioinformatics ecosystems, allowing users to integrate EpiFinder™ cNUC data seamlessly into their preferred analysis pipelines.

Flexible Access

Run the EpiFinder™ cNUC kit in-house or leverage Epigenica's analysis service for end-to-end support, from sample processing to data analysis.

Key Capabilities

- Parallel profiling of histone PTMs and DNA methylation directly from blood
- High-throughput generation of up to 144 genome-wide epigenetic datasets in a single run
- Cost-efficient multiplexed workflow that reduces sequencing burden and time to insight
- Simple, streamlined protocol with no DNA purification or spike-in controls required
- Seamless transition from sequencing to analysis-ready epigenomic data via an open-source pipeline

Validated Epigenomic Targets

EpiFinder™ cNUC includes a tailored, validated antibody panel, targeting key epigenetic features in transcriptional regulation and chromatin state. Histone modifications are profiled using antibody--based immunoprecipitation, while DNA methylation is captured using a tagged MBD2 protein.

The table below summarizes the core validated targets and their associated genomic features. Additional epigenetic marks have also been validated and are available upon request.

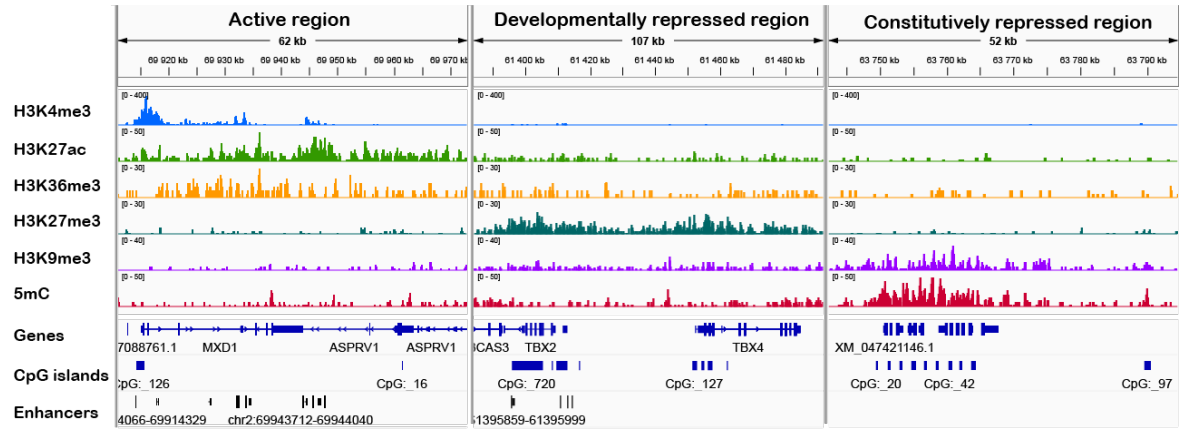
Target*	Associated genomic features
H3K4me3	Active and bivalent promoters
H3K27ac	Active promoters and enhancers
H3K36me3	Actively transcribed gene bodies, enriched towards the 3' ends of genes
H3K27me3	Facultatively repressed (Polycomb-associated) promoters and gene bodies
H3K9me3	Constitutively repressed regions and repetitive elements
5mC	DNA methylation, mediates gene repression at CpG islands

*Antibodies and capture proteins for all validated targets are provided with the kit.

Sensitivity and Resolution

Despite the limited number of circulating nucleosomes in liquid biopsy samples, EpiFinder™ cNUC captures genome-wide epigenetic information with high specificity. Data resolution is primarily determined by sample quality.

The figure below shows example genome browser (IGV) tracks from a healthy donor plasma sample displaying representative enrichment patterns for multiple epigenetic marks, including five histone modifications (H3K4me3, H3K27ac, H3K36me3, H3K27me3, H3K9me3) and DNA methylation (5mC). Distinct signal profiles are observed across active, developmentally repressed, and constitutively repressed genomic regions, consistent with their known regulatory functions.

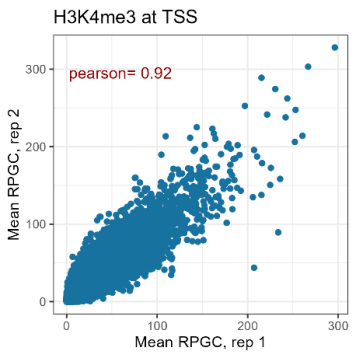


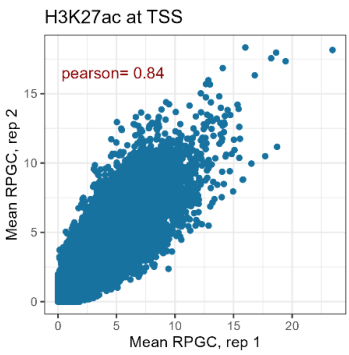
Note: Contact us for best-practice recommendations on blood collection and storage.

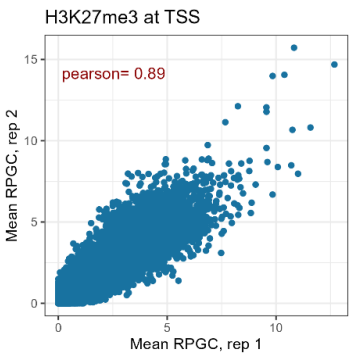
Reproducibility

EpiFinder™ cNUC delivers reproducible genome-wide epigenomic profiles from liquid biopsy samples. Strong concordance is observed across technical replicates for representative histone marks, demonstrating reliable performance.

The plots below show high agreement between technical replicates for H3K4me3, H3K27ac, and H3K27me3 generated from 1 mL of healthy EDTA plasma. Each data point represents the mean RPGC-normalized coverage at the transcription start site (TSS) of a protein-coding gene. High Pearson correlation coefficients confirm genome-wide reproducibility.







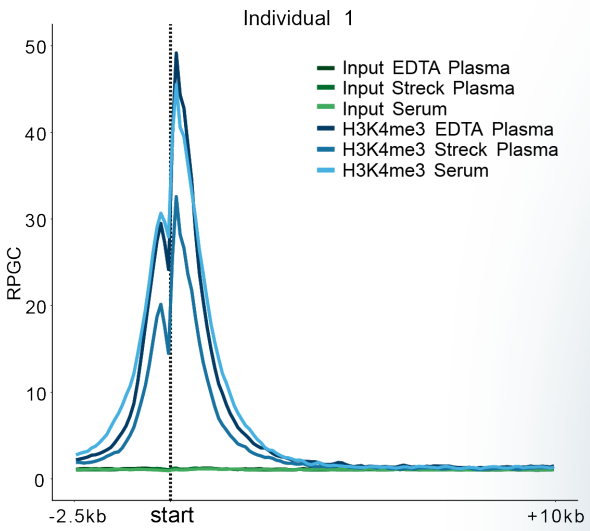
Validated Sample Types

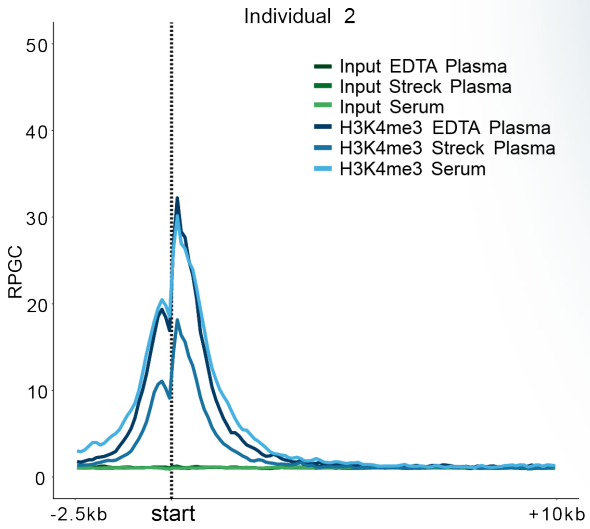
EpiFinder™ cNUC has been validated using:

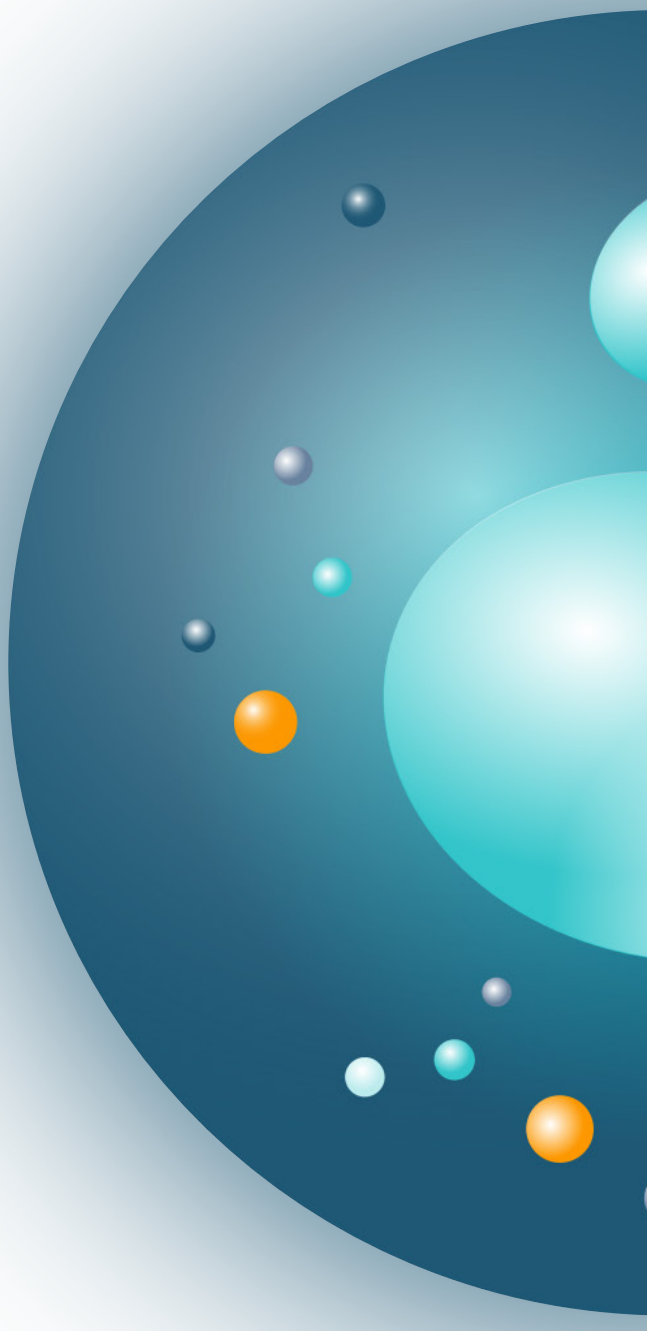
- Serum
- Plasma collected in EDTA tubes
- Plasma collected in Cell-Free DNA BCT® (Streck) tubes

The comparative data below demonstrate consistent H3K4me3 enrichment at transcription start site (TSS) regions from 100 µL serum or plasma samples collected from two individuals, using the validated tube types listed above.

H3K4me3 enrichment at TSS







Product Specifications

	EpiFinder™ cNUC
Profiling method	**Genome-wide**
Sample types	**Plasma or serum (see below)**
Conditions x targets	**24 samples x 6 targets**
Target	**Histone PTMs and DNA methylation**
Starting material per sample	**200 µl* to 1 ml**
Sequencing depth	**5-30 million reads/sample/IP**

* For input volumes below 1 mL, analysis service support is recommended.

The recommended starting volume for EpiFinder™ cNUC is 1 mL of plasma or serum. For studies with limited sample availability, lower input volumes can be accommodated through our Analysis Service. Our team will assess feasibility and provide guidance on expected data performance based on available sample volume.

Beyond Genetics: The Next Layer of Liquid Biopsy

EpiFinder™ cNUC brings epigenetic regulation into liquid biopsy. By enabling multiplexed, genome-wide profiling directly from plasma or serum, the platform expands analysis beyond genetic variation and unlocks regulatory information encoded in circulating nucleosomes. With scalable performance using standard plasma or serum volumes, EpiFinder™ cNUC establishes a new benchmark for liquid biopsy epigenomics.

Connect with our experts at support@epigenica.se
to learn more about EpiFinder™ cNUC.

www.epigenica.se

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