MassARRAY® - An Ideal Genetic Analysis System

EpiTYPER® DNA Methylation analysis is performed on Agena Bioscience’s MassARRAY® System, which uses MALDI-TOF mass spectrometry for robust and precise signal detection and quantification. The MassARRAY System can also be used for other nucleic acid analysis methods, including SNP and somatic mutation detection, and miRNA and CNV analysis. All methods offer flexible assay design, fast turn-time to results, and the ability to run from tens to thousands of samples daily. These features make it the ideal genetic analysis system for validation and fine mapping studies in basic and translational research settings.

The Complete Solution for Genomic Analysis

- HIGH PERFORMANCE: Accurate MALDI-TOF mass spectrometry detection provides unparalleled specificity and sensitivity for the most reliable results.
- MAXIMUM FLEXIBILITY: Analyze any combination of SNPs and samples to meet your study requirements.
- HIGHLY SCALABLE: 24-, 96-, and 384-well options for low to high-throughput applications.
- EASY TO USE: Data acquisition software streamlines your workflow, and robust assay design software automates primer design and optimization to maximize efficiency and minimize experimental variability.
- A VARIETY OF GENOMIC APPLICATIONS: Ready-to-use reagent sets for somatic mutation analysis, genotyping, methylation analysis, and quantitative applications (gene expression and copy number variation) provide a broad and flexible menu with short turnaround time.
- CHALLENGING SAMPLE TYPES: Due to the short amplicon lengths needed for the assays, virtually all DNA sample types are amenable to analysis on the MassARRAY System.
- HIGH PERFORMANCE: Accurate MALDI-TOF mass spectrometry detection provides unparalleled specificity and sensitivity for the most reliable results.
- MAXIMUM FLEXIBILITY: Analyze any combination of SNPs and samples to meet your study requirements.
- HIGHLY SCALABLE: 24-, 96-, and 384-well options for low to high-throughput applications.
- EASY TO USE: Data acquisition software streamlines your workflow, and robust assay design software automates primer design and optimization to maximize efficiency and minimize experimental variability.

Provides a complete system for discriminating methylated vs. non-methylated DNA, includes software, reagents, and MALDI-TOF mass spectrometry for detection and quantification. Integrate 10s-100s of samples and CGG sites in amplicons from 200 - 600 bp and detect down to 5% differences in methylation. Validate methylation array, nest gen sequencing, or gene promoter study results. Referenced in over 400 publications in diverse fields, including cancer, differentiation and development, diagnostics research, metabolism, and epigenetics.

Published Studies Using the MassARRAY System

Visit www.agenabioscience.com to search our online database for published studies using the MassARRAY System in your area of interest.

REFERENCE

Ordering Information

Reagent sets are available in 24-, 96-, and 384-well formats, and are designated for use with the MassARRAY System with EpiTYPER software.

### EpiTYPER COMPLETE REAGENT SETS

<table>
<thead>
<tr>
<th>CAT NO.</th>
<th>DESCRIPTION</th>
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### EpiTYPER ACCESSORY REAGENT SETS

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<tr>
<td>11324</td>
<td>PCR Accessory and Enzyme kit (LIBE machine)</td>
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<td>11320</td>
<td>PCR Accessory kit (LIBE machine)</td>
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EpiTYPER® DNA Methylation Analysis

Overview

Agena Bioscience’s DNA methylation analysis technology (EpiTYPER®) is one of the most reliable quantitative methods available today for analyzing DNA methylation changes.1,2 The technology, which has been referenced in more than 400 peer-reviewed journal articles, includes the following components:

- EpiDesigner - software for genomic target selection and PCR primer design.
- EpiQuik™ Bisulfite Kits - reagents and consumables for all downstream processes, following bisulfite treatment of DNA.

Benefits of the EpiTYPER DNA Methylation Analysis Technology

EFFICIENCY
- Genomic bisulfite-treated DNA to data in 8 hours.
- Covers multiple CpGs in amplicons of up to 600 bp.
- Compatible with many sample types, including formalin-fixed paraffin-embedded tissue.

PRECISION & ACCURACY
- High precision (5% CV).
- High inter-laboratory reproducibility.

SENSITIVE
- Detects down to 5% change in methylation levels.

How it Works

EpiTYPER biochemistry starts with bisulfite treatment of genomic DNA, followed by PCR amplification of target regions. The reverse primers contain a T7 promoter tag. Next, in vitro RNA transcription is performed, followed by base-specific RNA cleavage. Finally, the cleavage products are analyzed using MALDI-TOF mass spectrometry (MassARRAY® Analyzer). The methylated and non-methylated cytosine residues in the original genomic DNA are distinguished using EpiTYPER Software.

Workflow - From Assay Design to Results

STEP 1: ASSAY DESIGN

EpiDesigner is an online automated design tool for DNA methylation experiments, on the MassARRAY® System. Just enter your target sequences and the software determines primer designs for maximizing sequence coverage. In addition to optimized primer sequences, EpiDesigner delivers an easy-to-read graphical presentation of the amplification designed over your target regions, as well as annotating distinct CpG sites covered by the assays.

STEP 2: BISULFITE TREATMENT

Genomic DNA from all samples is extracted and treated with bisulfite. This treatment converts unmethylated cytosine residues into uracil (shown boxed in red), while methylated cytosine residues (blue) are unaffected. This step results in the generation of methylation-dependent sequence changes in the DNA template.

STEP 3: PCR, IN VITRO TRANSCRIPTION, AND RNA CLEAVAGE DESIGN

The EpiTYPER Assay starts with PCR using T7 promoter-tagged reverse primers to amplify the target regions while preserving the bisulfite-induced sequence changes. After SAP treatment, in vitro transcription is performed and the resulting RNA transcripts are specifically cleaved at uracil residues. The resulting fragments differ in size and mass, depending on the sequence changes generated through bisulfite treatment. This difference allows the data analysis software to generate quantitative information for each analyzed target fragment.

STEP 4: DATA ACQUISITION AND ANALYSIS

The EpiTYPER reaction products are dispensed onto a SpectroCHIP® Array (CYPHER®). The Chip is then placed in the MALDI-TOF mass spectrometer for data acquisition, which typically requires 15-60 minutes. The results are automatically loaded into a database for data analysis with EpiTYPER software.

For Research Use Only. Not for use in diagnostic procedures.

EpiTYPER Software

The EpiTYPER software provides an advanced and convenient solution for the quantitative analysis of CpG methylation. Numerical and graphical interpretation tools are available and the data are automatically matched to the provided sequence. Basic statistical analysis and confidence ratings are available for built in quality control.

DATA ANALYZER MODE

Data Set Selection
- Clicking on any one of these buttons will determine which data set is included in tab panels and Epigram.

Spectrum Pane
- Automatically displays the mass spectrum for all analyzed amplicons and identifies the selected CpG (red arrow in spectrum).

Methylation Pane
- Obtain customized visualization of amplicon data in the most useful format for your needs.

Sequence View Pane
- This slider display shows the entire forward and reverse nucleotide sequence for the selected amplicon.

CUSTOM VIEWS OF AMPICONS

Display Options
- These menus allow a fine-tuning of program parameters, panels to display, and methylation color coding of CpG sites.

Epigram Pane
- This pane provides graphical representations of the CpG sites within the selected amplicon. Each is color-coded to represent the degree of methylation, providing a quick, reliable comparison between samples and CpG sites.
Overview
Agena Biosciences’ DNA methylation analysis technology (EpiTYPER) is one of the most reliable quantitative methods available today for analyzing DNA methylation changes. The technology, which has been referenced in more than 400 peer-reviewed journal articles, includes the following components:

- **EpiDesigner** - software for genomic target selection and PCR primer design.
- **EpiPT** (DnaCode) - reagents and consumables for all downstream processes, following bisulfite treatment of DNA.

Benefits of the EpiTYPER DNA Methylation Analysis Technology

**EFFICIENCY**
- Genomic DNA is extracted from bisulfite-treated DNA to use in 8 hours.
- Covers multiple CpGs in amplicons of up to 600 bp.
- Compatible with many sample types, including formalin-fixed paraffin-embedded tissue.

**PRECISE & ACCURATE**
- High precision (5% CV).
- High inter-laboratory reproducibility.

**SENSITIVE**
- Detects down to 5% change in methylation levels.

How it Works
EpiTYPER biochemistry starts with bisulfite treatment of genomic DNA, followed by PCR amplification of target regions. The reverse primers contain a TT promoter tag next to an RNA transcription signal. The resulting PCR products are then treated with T7 RNA polymerase (EpiTYPER Reaction). The methylated and non-methylated cytosine residues in the original genomic DNA are easily distinguished using EpiTYPER Software.

Workflow - From Assay Design to Results

**STEP 1: ASSAY DESIGN**
EpiDesigner is an online automated design tool for DNA methylation experiments, on the MassARRAY System. Just enter your target sequences and the software determines primer designs for maximizing coverage. In addition to optimized primer sequences, EpiDesigner delivers an easy-to-read graphical presentation of the amplification designed over your target regions, as well as annotating distinct CpG sites covered by the assays.

**STEP 2: BISULFITE TREATMENT**
Genomic DNA from all samples is extracted and treated with bisulfite. This treatment converts any non-methylated cytosine residues into uracil (shown below in red), while methylated cytosine residues (blue) are unaffected. This step results in generation of methylation-dependent sequence changes in the DNA template.

**STEP 3: PCR, IN VITRO TRANSCRIPTION, AND RNA CLEARANCE DESIGN**
EpiTYPER Assay starts with PCR using TT promoter-tagged reverse primers to amplify the target regions while preserving the bisulfite-induced sequence changes. After SAP treatment, in vitro transcription is performed and the resulting RNA transcripts are specifically cleaved at uracil residues. The resulting fragments differ in size and mass, depending on the sequence changes generated through bisulfite treatment. This difference allows the data analysis software to generate quantitative information for each analyzed target fragment.

**STEP 4: DATA ACQUISITION AND ANALYSIS**
The EpiTYPER reaction products are dispersed into a SpectrCHIP Array (EpiChip). The chip is then placed in the MALDI-TOF mass spectrometer for data acquisition, which typically requires 15-60 minutes. The results are automatically loaded into a database for data analysis with EpiTYPER software.

EpiTYPER Software
The EpiTYPER software provides an advanced and convenient solution for the quantitative analysis of CpG methylation. Numerical and graphical interpretation tools are available and the data are automatically matched to the provided sequence. Basic statistical analysis and confidence ratings are available for built-in quality control.

DATA ANALYZER MODE
Data Set Selection
- Clicking on one or both of these buttons will determine which data set is included in tab panels and Epigram.

Sequence View Pane
- This slider display shows the entire forward and reverse nucleotide sequence for the selected amplicon.

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Display Options
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- **EpiDesigner** - software for genomic target selection and PCR primer design.
- **EpiTYPER** - beacon-based reagents and consumables for all downstream processes, following bisulfite treatment of DNA.
- **MassARRAY** Analysis 4 - MALDI-TOF mass spectrometer for robust and precise signal detection and quantification.
- **EpiTYPER** Reporting Software - for data analysis and graphical presentation of the level of methylation at each CpG site in each sample.

Benefits of the EpiTYPER DNA Methylation Analysis Technology

**EFFICIENCY**
- Quick bisulfite-treated DNA to data in 8 hours.
- Covers multiple CpGs in amplions of up to 600 bp.
- Compatible with many sample types, including formalin-fixed paraffin-embedded tissue.

**PRECISE & ACCURATE**
- High precision (5% CV).
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- Detects down to 5% change in methylation levels.

How it Works

EpiTYPER biochemistry starts with bisulfite treatment of genomic DNA, followed by PCR amplification of target regions. The reverse primers contain a T7 promoter tag next to the bisulfite-induced sequence changes. After SAP treatment, the DNA templates are amplified with tag-specific primers that detect the original unmodified bases. After PCR, in vitro transcription is performed and the resulting RNA transcripts are specifically cleaved at uracil residues. The resulting fragments differ in size and mass, depending on the sequence changes generated through bisulfite treatment. This difference allows the data analysis software to generate quantitative information for each analyzed target fragment.

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**Step 2: Bisulfite Treatment**

Genomic DNA from all samples is extracted and treated with bisulfite. This treatment converts any non-methylated cytosine residues into uracil (shown in red), while methylated cytosine residues (blue) are unaffected. This step results in the generation of methylation-dependent sequence changes in the DNA template.

**Step 3: PCR, In Vitro Transcription, and RNA Cleavage Design**

The EpiTYPER Assay starts with PCR using T7-promoter-tagged reverse primers to amplify the target regions while preserving the bisulfite-induced sequence changes. After SAP treatment, in vitro transcription is performed and the resulting RNA transcripts are specifically cleaved at uracil residues. The resulting fragments differ in size and mass, depending on the sequence changes generated through bisulfite treatment. This difference allows the data analysis software to generate quantitative information for each analyzed target fragment.

**Step 4: Data Acquisition and Analysis**

The EpiTYPER reaction products are dispersed onto a SpectroCHIP Array (Chip). The Chip is then placed in the MALDI-TOF mass spectrometer for data acquisition, which typically requires 55-60 minutes. The results are automatically loaded into a database for data analysis with EpiTYPER software.

**Workflow - From Assay Design to Results**

**EpiTYPER Software**

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MassARRAY System – An Ideal Genetic Analysis System

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Published Studies Using the MassARRAY System

Visit www.agenabioscience.com to search our online database for published studies using the MassARRAY System in your area of interest.

**REFERENCE**

1. A systematic comparison of quantitative high-resolution DNA methylation analysis and methylation-specific PCR (MSP) for CpG island hypermethylation.
2. Quantitative high-resolution analysis of DNA methylation using a novel system of microfluidics and mass spectrometry.

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EpiTYPER® DNA Methylation Analysis

A complete system for discriminating methylated vs. non-methylated DNA, includes software, reagents, and MALDI-TOF mass spectrometry for detection and quantification.

- Characterize 10s-100s of samples and CG sites in amplicons from 200 - 600 bp and detect down to 5% differences in methylation.
- Validate methylation array next gen sequencing, or gene promoter study results.
- Referenced in over 400 publications in diverse fields, including cancer, differentiation and development, epigenetics, and diabetes.

High-Resolution Quantitative Methylation Profiling with EpiTYPER® and the MassARRAY® System

EpiTYPER Complete Reagent Sets

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EpiTYPER Accessory Reagents

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- **CHALLENGING SAMPLE TYPES**
  - Due to the short amplicon lengths needed for the assays, virtually all DNA sample types are amenable to analysis on the MassARRAY® System.

- **HIGH PERFORMANCE**
  - EpiTYPER® assay accuracy and sensitivity for the most reliable results.
- **MAXIMUM FLEXIBILITY**
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- Validate methylation array, next gen sequencing, or gene promoter study results.
- Referenced in over 400 publications in diverse fields, including cancer, differentiation and development, diagnostics research, metabolism, and epigenetics.

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<td>MassCLEAVE T7 Kit</td>
<td>100 x 24 Reagents only</td>
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<td>PCR Accessory and Enzyme Set (100 mL machine)</td>
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<td>PCR Accessory Set</td>
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<td>01736</td>
<td>PCR Enzyme (100 mL machine)</td>
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<td>EZ2 DNA-Plus Bioblot Treatment Kit</td>
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<td>EZ2 DNA-Plus Bioblot Treatment Kit</td>
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### Published Studies Using the MassARRAY® System

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**REFERENCE**


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