Methylamp™ MS-qPCR Fast Kit

Base Catalog # P-1028

PLEASE READ THIS ENTIRE USER GUIDE BEFORE USE

Uses: The Methylamp™ MS-qPCR Fast Kit is very suitable for quantitative methylation-specific PCR in a fast format using very minute amounts of DNA.

When using the Methylamp™ MS-qPCR Fast Kit for MS-qPCR with very small amounts of input DNA (< 50 pg), the number of PCR cycles should be greater than 45.

Control reactions should be performed to ensure that the PCR primers are specific to bisulfite-converted DNA and methylated DNA. For performing control reactions, methylated bisulfite converted DNA and unconverted genomic DNA are required. A pair of β-actin primers (for human) specifically for bisulfite converted DNA is included in the kit for determining the bisulfite conversion efficiency of bisulfite reactions by using both bisulfite converted DNA and unconverted genomic DNA.

Precautions: For lowering the risk of contamination during the PCR, it is recommended to wear fresh gloves and use pipette tips with aerosol filters for the reaction setup.

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KIT CONTENTS

Component	100 reactions Cat. #P-1028-100	200 reactions Cat. #P-1028-200
Master Mix (2X)	1 vial (1.0 ml each)	2 vials (1.0 ml each)
DNA/RNA-free Water	1 vial (1.2 ml each)	2 vials (1.2 ml each)
Control Primers (β-actin)		
Forward (100 µM)	8 µl	20 µl
Reverse (100 µM)	8 µl	20 µl
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SHIPPING & STORAGE

The Methylamp™ MS-qPCR Fast Kit is shipped on ice packs. The kit should be stored immediately at –20°C upon receipt.

The kit is stable for at least 6 months from the shipment date, when stored properly.

MATERIALS REQUIRED BUT NOT SUPPLIED

- ☐ Real-time PCR instrument
- ☐ PCR reaction plates or thin-wall PCR tubes
- ☐ Pipette and pipette tips

GENERAL PRODUCT INFORMATION

Quality Control: Each lot of the Methylamp™ MS-qPCR Fast 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 call 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.

Usage Limitation: The Methylamp™ MS-qPCR Fast Kit is for research use only and is not intended for diagnostic or therapeutic applications.

A BRIEF OVERVIEW

DNA methylation plays an important role in the regulation of gene expression, tumorigenesis, and other genetic and epigenetic diseases. Thus, the detection of methylation in some genes of diseased cells could provide very useful information for discrimination of that disease. Many methods for the gene/sequence-specific detection of DNA methylation have been developed over the recent years, of which methylation-specific PCR (MS-PCR) is the most commonly used. MS-PCR, through bisulfite conversion of cytosine to uracil in DNA, is able to selectively amplify the methylated sequences with primers specific for methylation.

A challenge resides in the MS-PCR amplification of the bisulfite-treated DNA. The high redundancy of the target sequence due to the original GC richness creates long stretches of thymines, which are often difficult to read for the polymerases. Furthermore, amplification is difficult due to the necessity of extended time to amplify bisulfite-treated DNA and the reduced primer binding after the chemical conversion.

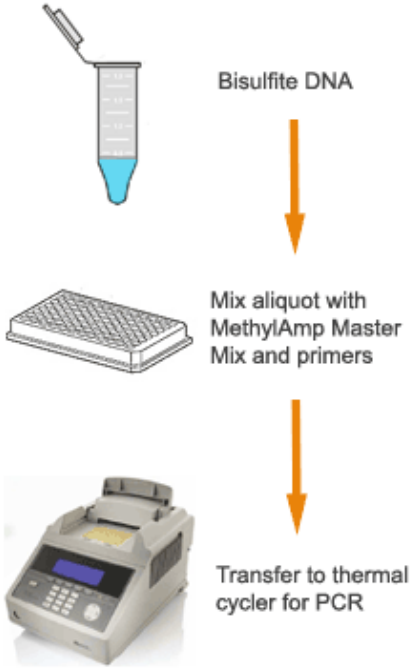
Epigentek provides the Methylamp™ MS-qPCR Fast Kit to address these issues. This kit allows for a fast, specific, sensitive and reproducible methylation-specific quantitative PCR to be processed. The novel hot start DNA polymerase contained in this kit can specifically reduce the overall time required for MS-qPCR from approximately 2.5 hours to just 70 minutes. The kit also facilitates sensitivity and specificity of MS-qPCR by significantly increasing primer-bisulfite DNA template annealing, while simultaneously reducing non-specific annealing. This results in considerable time savings and a more efficient MS-qPCR.

The Methylamp™ MS-qPCR Fast Kit has the following features:

- An extremely fast procedure that can be finished within 70 minutes.
- Abundant yields due to high amplification efficiency.
- Highly accurate and specific in MSP, with reduced false-positive results.
- Convenient master mix format to allow easy reaction setup.
- Simple, reliable, and consistent assay conditions.
- Can be used with any block-based real-time PCR instrument.

PRINCIPLE & PROCEDURE

The Methylamp™ MS-qPCR Fast Kit provides a master mix format, which contains a hot start DNA polymerase, dNTPs, an MS-PCR enhancer, an optimized buffer, and an intercalating green dye. This master mix allows for a convenient and easy reaction setup. The unique hot start DNA polymerase is provided in an inactive state at ambient temperature and is reactivated by several minute incubations at 95˚C, which can easily be integrated into existing thermal cycling steps. The hot start DNA polymerase in combination with the optimized buffer ensures the MS-qPCR specificity and sensitivity. The green dye allows for DNA detection and analysis without using a sequence-specific probe.



Schematic procedure for using the Methylamp™ MS-qPCR Fast Kit.

PROTOCOL

Prepare the PCR Reactions

1. Thaw all reaction components including **Master Mix**, **DNA/RNA-free Water**, **Primer** solutions, and DNA template. Mix well by vortexing briefly. Keep components on ice while in use, and return to –20°C immediately following use.

2. Add components into each well according to the following procedures:

Component	Size (µl)	Final Concentration
Methylamp Master Mix (2X)	10 µl	1X
Forward Primer	1 µl	0.4-0.5 µM
Reverse Primer	1 µl	0.4-0.5 µM
DNA Template	1-2 µl	50 pg-0.1 µg
DNA/RNA-free H_2O	6-7 µl	
Total Volume	**20 µl**	

Note: For the Negative Control, use DNA/RNA-free water instead of DNA template.

Program the PCR Reactions

3. Place the reaction plate in the instrument.

4. Set the PCR conditions as follows:

Cycle Step	Temperature	Time	Cycle

Activation	95°C	7 min	1
Cycling	95°C 55°C 72°C	10 sec 10 sec 8 sec	40-45
Final Extension	72°	1 min	1

TROUBLESHOOTING

Problem	Possible Causes	Suggestions
Little or No Amplification Product	Primer is incorrect.	Check primer design. If it is specifically for bisulfite-treated DNA, confirm the accuracy of the sequence information. Redesign the primers according to the guidance of the MS-PCR primer design or by using MethPrimer.
	Primer is degraded or primer concentration is not optimal.	Repeat PCR with different primer concentration by 0.1 µM increments. Check for possible degradation of the primers on a denaturing polyacrylamide gel.
	Bisulfite treatment of DNA is poor or input bisulfite DNA is insufficient.	Check PCR specificy and reliability with β-actin control primers (human) included in the kit. Increase the amount of input bisulfite DNA. If necessary, make new bisulfite DNA and repeat PCR with new template. Note: For non-human samples, the user may design their own β-actin primers or contact Epigentek for further assistance.
	DNA is degraded.	Check if DNA is degraded prior to or after DNA modification.
	Incorrect PCR program including insufficient denaturing, annealing & extension time/temperature, and insufficient number of cycles.	Check if the denaturing, annealing, and extension time/temperature programming is correct. Increase number of cycles.
	Hot-start DNA polymerase is not activated.	Perform initial enzyme activation for 7-9 minutes at 95°C.
	Pipetting error or missing reagents.	Check the concentrations and storage conditions of the reagents including primers. Ensure the ratio of master mix to primer-template is 1:1.
	Instrument and PCR plate problem.	Check the power to the real-time PCR instrument and ensure that the PCR plate fits in the instrument.
Non-Specific Amplification	Number of PCR cycles is too high.	Reduce the number of cycles to eliminate non-specific PCR.

Product	Template concentration is too high.	When amplifying bisulfite DNA, the initial concentration of template in the reaction mixture should not exceed 100 ng per 20 µl of reaction volume.
	Insufficient bisulfite treatment of DNA.	Check PCR specificity and reliability with β-actin primer included in the kit. If necessary, make new bisulfite DNA and repeat PCR.
	Primer design is not optimal.	Check primer design. If it is specifically for bisulfite-treated DNA, confirm the accuracy of the sequence in formation. Redesign the primers according to the guidance of the MS-PCR primer design or by using MethPrimer.

RELATED PRODUCTS

DNA Bisulfite Modification

P-1026 BisulFlash™ DNA Modification Kit
P-1008 Methylamp™-96 DNA Modification Kit
P-1016 Methylamp™ Whole Cell Bisulfite Modification Kit
P-1002 Methylamp™ Coupled DNA Isolation & Modification Kit

DNA Methylation Analysis

P-1005 TuMinute™ PCR Clean-Up Kit
P-1011 Methylamp™ Universal Methylated DNA Kit
P-1019 Methylamp™ Universal Methylated DNA Preparation Kit

DNA Methylation Quantification

P-1014B Methylamp™ Global DNA Methylation Quantification Ultra Kit
P-1021 SuperSense™ Methylated DNA Quantification Kit

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