MGMT Methylation Detection Kit

Real-Time PCR assay

EntroGen
Automation • Reagents • Software

- Detects and quantifies MGMT promoter methylation
- Bisulfite conversion kit included
- 5% Methylation Reference Control available to benchmark during bisulfite conversion process
- 44 tests per kit
- Available as RUO (US) / IVD (EU)

INTENDED USE:

The MGMT Methylation Detection Kit is a methylation specific PCR (MSP) assay intended for the detection of O (6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation in genomic DNA of glioblastoma multiform (GBM) tumors. MGMT promoter methylation has been shown to lead to increased sensitivity to alkylating agents such as temozolomide and has favorable prognostic significance. For research use only in the U.S. Not for use in diagnostic procedures. For in vitro diagnostic use in the European Union.

FOR USE WITH:

- Applied Biosystems® 7500/7500 Fast, QuantStudio™ 5
- Bio-Rad - CFX96
- Qiagen - Rotor-Gene® Q
- Roche - LightCycler® 480

PACKAGE CONTENTS

The MGMT Methylation Detection Kit is provided in two (2) boxes.
The Bisulfite Conversion Kit (BCK-RT50 kit) contains reagents sufficient for the bisulfite conversion of 50 samples.

Description	Tubes
Mutation Detection Reaction Mix (2X)	2
MGMT Detection Primer Mix	1
MGMT Standards 1, 2, and 7	3
MGMT Standard 3	1
Control Standard Diluent	1
Carrier RNA	1
Bisulfite Solution	3
DNA Protect Buffer	1
RNase-Free Water	1
DNA Spin Columns	50
Binding Buffer	1
Wash Buffer (concentrate)	2
Desulfonation Buffer (concentrate)	1
Elution Buffer	1

MGMT-RT44 provides materials sufficient for 60 reactions per Primer Mix when standards and samples are prepared in triplicate. Standards and samples must be set up in triplicate. With this configuration, the kit contains enough reagents to process 44 samples across two runs.

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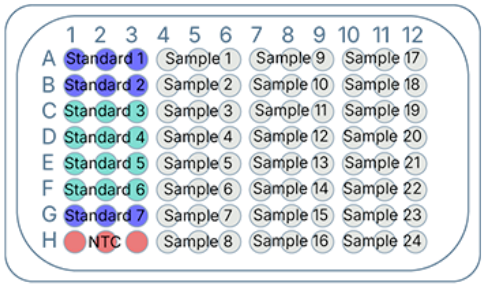
Mutation Detection Reaction Mix (2X)	2
MGMT Detection Primer Mix	1
MGMT Standards 1, 2, and 7	3
MGMT Standard 3	1
Control Standard Diluent	1

PLATE SETUP

A single 96-well PCR plate can accommodate up to 24 unknown samples



PRINCIPLE OF THE ASSAY

The MGMT Methylation Detection Kit is a quantitative real-time PCR assays designed to distinguish between methylated and non-methylated cytosines in the MGMT gene DMR2 region aligned to Hg38: chr10: 129,467,243 –129,467,365 and covers CpG sites 75-86.
The assays use specific primers to amplify only methylated MGMT CpG sites in the DMR2 region in bisulfite treated DNA samples. They also amplify an endogenous control gene (Actin B) in relation to which the extent of MGMT promoter methylation is calculated.
Detection of these amplified regions is enabled by specific fluorescent hydrolysis probes, complementary to the methylated MGMT DMR2 region (labeled with the FAM fluorophore), and the endogenous control gene (tagged with the VIC fluorophore).
MGMTX-RT44 provides a comprehensive suite for bisulfite conversion and MGMT methylation detection. MGMTX-RT44 includes bisulfite conversion reagents with optimized bisulfite mix, DNA Protection Buffer with pH indicator, as well as specific reaction conditions and cycling program, for efficient conversion of unmethylated cytosines to uracils, while minimizing DNA fragmentation.
The testing procedure involves the following simple steps:

1. Isolation of DNA from tumor biopsies, paraffin-embedded sections (FFPE), or fresh frozen tumors.
2. Bisulfite conversion of samples (e.g., isolated DNA, methylated and unmethylated controls) using the provided reagents in MGMTX-RT44.
   a. Treatment of DNA with bisulfite solution.
   b. Binding of the resulting ssDNA to a DNA Spin Column membrane, followed by wash steps.
   c. On-column desulfonation step.
   d. On-column DNA washes to remove desulfonation reagent and stop further DNA damage.
   e. Elution of DNA for the downstream real-time PCR assay.
3. Amplification of treated DNA using the provided reagents in the MGMT Methylation Detection kit.
4. Data analysis and interpretation using the real-time PCR software

HANDLING AND STORAGE

The MGMT Methylation Detection Kit includes frozen PCR reagents and bisulfite conversion components with reagent-specific storage requirements. Complete storage, handling, and stability information is provided in the Instructions for Use.

ASSAY PERFORMANCE

Limit of Blank

The performance of the MGMT Methylation Detection Kit in the absence of template and with unmethylated specimens at 2 different input (200 ng and 500 ng DNA into the EZ DNA Methylation-Lightning™ Kit, 30µl elution volume) was assessed on a CFX96 instrument to determine the level of background noise for unmethylated DNA. Nine blank replicates were run per input (0 ng, 200 ng, 500 ng) on 7 different plates for a total of 63 replicates per input. No signal was detected in any of the replicates.

Limit of Quantification

Methylated brain gDNA was diluted from 100% methylated down to 3.125% methylated in a background of unmethylated brain gDNA. The assay was performed on a CFX96 instrument 6 times in triplicate for each dilution and at 3 different inputs – 100 ng (~850 ACTB copies), 250 ng (~3100 ACTB copies) and 500 ng (~4600 ACTB copies). The different inputs refer to the load in the upstream EZ DNA Methylation-Lightning™ Kit. The Limit of Quantification (LoQ) was determined to be 6.25% methylation at all inputs (100 ng, 250 ng and 500 ng).

Precision

Precision of the assay was established on a CFX96 instrument by performing the assay on serial dilution of 100% methylated brain gDNA in a background of unmethylated brain gDNA. Independent dilution batches for different inputs, as well as three different lots of the reagents were used to run the assay. The table below shows the average percent methylation from 6 runs of triplicates for dilutions at the 100 ng input (DNA input into the EZ DNA Methylation-Lightning™ Kit, 30 µl elution volume).

Dilution	Target % Methylation of Dilutions	Mean % Methylation	Stdev [%]
1:2	50%	45.43%	2.37
1:4	25%	24.98%	1.32
1:8	12.5%	11.78%	0.67
1:16	6.25%	5.92%	0.40
1:32	3.125%	2.52%	0.33

Accuracy

Accuracy of the assay was established by testing 12 DNA samples isolated from FFPE sections of gliomas that had been screened for MGMT promoter methylation using EPIC array. EPIC array is not a quantitative assay and only distinguishes between methylated versus non-methylated, therefore only this metric was used for accuracy testing. The methylated cut-off for the MGMT Methylation Detection Kit was >0%. The results between both assays were concordant for all 12 samples, with 6 non-methylated and 6 methylated samples.

CONTACT

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For research use (RUO) in the U.S
For in vitro diagnostic use (IVD) in the European Union