human MGMT PCR Primer Pair™

Catalog Number: RDP-126-025
Pack Size: 25 tests
Lot Number:
Expiration Date:

Specifications and Use

Description

- Human MGMT (O-6 methylguanine-DNA methyltransferase) specific PCR* primers for RT-PCR (reverse transcription followed by polymerase chain reaction) analysis of mRNA expression.

Components

- **Primer Pair**- Lyophilized. Each vial contains 375 pmoles of each primer. Adjust to a final concentration of 7.5 pmoles/µL by resuspending Primer Pair in either 50 µL autoclaved deionized water or TE buffer (10 mM Tris HCl, pH 8.0 @ 25° C; 1 mM EDTA, pH 8.0 @ 25° C).
- **Positive Control 18** - Lyophilized. Each vial contains 150 ng synthetic double-stranded DNA. Resuspend in 30 µL autoclaved deionized water or TE buffer. Not intended for quantitative purposes.

Sequence

- GenBank® NM_002412

Product Sizes

- **cDNA**: 283 base pairs (bp)
- **Genomic DNA**: Partial sequence available. No product observed.
- **Positive Control 18**: 350 bp
- **Alternate splice variants**: None Reported

Storage

- The lyophilized Primer Pair is stable for greater than one year at -20° C. The Primer Pair resuspended in water or TE buffer is stable for one year at -20° C in a non-frost free freezer. Aliquot in single use portions.
- **Avoid repeated freeze-thaw cycles.**

Tips

- Use either random primers or oligo (dT) for RT.
- The recommended annealing temperature is 55° C.

Genbank is a registered trademark of the United States Department of Health and Human Services.
* PCR is covered by US Patent Nos. 4683195 and 4683202 assigned to Hoffmann-La Roche.

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PCR Guide

We recommend the following protocol for R&D Systems' PCR Primer Pairs™.

1. Prepare on ice a PCR master mix containing the following components for each reaction:

5 µL	10x dNTP mix (10x= 2 mM each)
5 µL	10x Taq buffer (with $MgCl_2$)
0.5 µL	Taq DNA Polymerase (5 units/µL)
36.5 µL	autoclaved deionized water

2. Pipette 47 µL of the master mix into a PCR tube and add:
   2 µL primer mix
   1 µL cDNA template or 1 µL PCR Positive Control.

3. Briefly spin each tube and add 30 µL mineral oil to each reaction to prevent evaporation. Cap the tube and place in a thermocycler.

4. Perform the following program:
   94 °C for 4 minutes

   94 °C for 45 seconds
   55 °C for 45 seconds
   72 °C for 45 seconds
   } x 30-35 cycles

   72 °C for 10 minutes

Analysis of Results: The PCR products can be analyzed by agarose gel electrophoresis. For predicted sizes of PCR products, refer to the specification sheet.

Troubleshooting

Problem	Suggestions
No PCR products obtained	• Operator error may have occurred during reaction assembly. Run positive control reaction. • Unsuccessful cDNA synthesis. Repeat PCR reaction with primers for a housekeeping gene as a control to show that RT was successful. • RNA may be degraded. Check the integrity of RNA by gel electrophoresis. A good quality RNA preparation should show the 28S and 18S ribosomal RNAs in an approximate 2:1 ratio. If RNA appears degraded, repeat RNA isolation. • RNA secondary structure may be inhibiting cDNA synthesis. This can often be overcome by using random primers. • The target RNA concentration may be too low. Increase the amount of cDNA template used in PCR reaction or the number of cycles used to amplify the cDNA.
Smearing or bands of unpredicted size	• Non-specific priming may have occurred. Increase annealing temperature, perform a hot-start or decrease the amount of cDNA template.