



AmpliSNiP *MTHFR* rs1801133 (qPCR)

Diagnostic kit for analysis of the **rs1801133** polymorphism located in the ***MTHFR*** gene by the **DMAS-qPCR** technique

Cat. No.	Quantity
SNP008-50	50 assays

The diagnostic kit is intended for professional use in specialised clinical, diagnostic and research laboratories. We strongly recommend that you read the user manual attached to the diagnostic kit before first use.

TRANSPORT

The **AmpliSNiP MTHFR rs1801133 (qPCR)** kit is transported in dry ice. Immediately unpack the kit after delivery. Immediately inform the manufacturer if the tape securing the transport box or the seal of the commercial packaging are damaged or there is no dry ice left in the styrofoam transport box.

KIT CONTENTS

<i>Component</i>	<i>Description</i>	SNP008-50 <i>50 assays</i>	<i>Tube lid colour</i>
RM-T	Reaction Mixture	1 × 550 µL	Green
RM-C	Reaction Mixture	1 × 550 µL	Green
PC-T	Positive Control	1 × 300 µL	Red
PC-C	Positive Control	1 × 300 µL	Red
WATER	Water	1 × 1500 µL	White

STORAGE AND TRANSPORTATION CONDITIONS

- Store the kit components at -20°C.
- **AVOID THE EXPOSURE OF THE RM COMPONENT TO THE LIGHT.**
- Avoid repeated freezing/thawing cycles of the kit components (especially the RM component). The kit is stable after 10 repeated freezing/thawing cycles.
- The kit is stable under recommended storage conditions until the expiry date stated on the label.
- Do not use the kit components after the expiry date.

PRODUCT DESCRIPTION

Application

The **AmpliSNiP MTHFR rs1801133 (qPCR)** kit is designed to allow the analysis of the rs1801133 polymorphism located in the *MTHFR* gene, which encodes an enzyme involved in the folic acid metabolism. The rs1801133 polymorphism has two allelic versions: T and C. The rs1801133(T) allelic variant encodes less active methylenetetrahydrofolate reductase. The diagnostic kit is intended for *in vitro* diagnostics. The kit is a qualitative type: it is used to determine the allelic variants of the human gene.

Method principle

The **AmpliSNiP MTHFR rs1801133 (qPCR)** kit is based on the DMAS-qPCR (double – mismatch allele – specific qPCR) technology. The individual gene variants (alleles) are determined by specific primers systems. These primers allow amplification of a fragment of human DNA depending on the DNA sequence at the polymorphic site. Accumulating amplicons (the amplified fragments of human DNA) are detected owing to specific TaqMan[®] probe which is hydrolysed during DNA synthesis. After hydrolysis, the FAM fluorescent dye is released from the probe, which is detected by the optical system of the Real Time PCR device. The **AmpliSNiP MTHFR rs1801133 (qPCR)** kit contains two **RM** components allowing detection of the C or T nucleotide within the polymorphic site (**RM-C** and **RM-T**, respectively).

To increase the reliability of the results, the **RM** components of the **AmpliSNiP MTHFR rs1801133 (qPCR)** kit contain an internal control system, a probe and primers system for amplification and detection of the sequence specific for human DNA. Detection of the human DNA occurs due to the release of the HEX fluorescent dye from the probe. The use of the internal control allows monitoring of the addition of human DNA to the reaction.

The **AmpliSNiP MTHFR rs1801133 (qPCR)** kit contains **PC** (positive control) components which include DNA molecules encoding both versions of the *MTHFR* gene. Real-time PCR reactions with **PC** components

(**PC-C** and **PC-T**) were designed to serve as a negative control for human genomic DNA contamination. The proper course of the reaction with the **PC-C** and **PC-T** components should provide a positive result in the FAM channel (detection of *MTHFR* variants) and a negative result in the HEX channel indicating no human genomic DNA contamination.

ADDITIONAL INSTRUMENTS AND MATERIALS

The kit allows a complete diagnostic procedure (analysis of the rs1801133 polymorphism within the *MTHFR* gene in samples of human origin) **only** in conjunction with the kit for DNA extraction from the sample and the Real Time PCR device.

NOTE 1: The **AmpliSNIp *MTHFR* rs1801133 (qPCR)** kit is compatible with Real Time PCR devices having detection of HEX and FAM fluorescent dyes;

A full list of compatible Real Time PCR devices can be found on the manufacturer's website.

NOTE 2: The **AmpliSNIp *MTHFR* rs1801133 (qPCR)** kit does not contain the ROX reference dye.

For devices requiring normalisation, the ROX dye should be added to the RM component at the appropriate concentration. The ROX dye is not included in the kit.

TECHNICAL SPECIFICATION

Quality control	Complies with ISO 13485: Medical devices - Quality management systems
Optimal amount of DNA added to the reaction	to 100 ng

DNA EXTRACTION

For DNA extraction and purification the silica-based DNA extraction kits are recommended. These kits ensure good-quality of DNA samples. DNA samples should be stored at 2–8°C (short storage period), at -20°C or at a lower temperature (long storage period). The amount of the material required depends on DNA extraction method.

NOTE 3: Samples should be collected in sterile tubes. Prior to DNA extraction, samples must be stored for a period and under conditions that guarantee the stability of the human genetic material.

NOTE 4: Other DNA extraction methods can cause contamination of the DNA samples with compounds that significantly reduce the efficiency of the PCR reaction. This leads to lower sensitivity of the assay and, in extreme situations, complete inhibition of DNA amplification.

REAL TIME PCR REACTION

1. Determine the number of DNA samples to be analysed (**n**).
2. Thaw the kit components. After thawing, mix the tubes thoroughly and briefly spin them.

Store thawed components at 2–8°C or in ice.

AVOID THE EXPOSURE OF THE RM COMPONENT TO THE LIGHT.

3. Determine the amount of the DNA sample added to the reaction (**x** µL).

NOTE 5: Optimal amount of DNA added to the reaction should not exceed 100 ng. A large amount of genomic DNA added to the PCR reaction mixture can lead to non-specific reactions.

4. Prepare two reaction mixtures by mixing the **RM** component and water in the proportions given below:

	Components		
	RM-C	RM-T	Water
1	$(n + 2) \times 11 \mu\text{L}$		$(n + 2) \times (9 - x) \mu\text{L}$
2		$(n + 2) \times 11 \mu\text{L}$	$(n + 2) \times (9 - x) \mu\text{L}$

- Add $(20 - x) \mu\text{L}$ to each of the mixtures to $n + 1$ reaction tubes.
- Add $x \mu\text{L}$ of the DNA samples to n reaction tubes. Each DNA sample should be added to each of the two tubes containing different reaction mixtures (**RM-C** and **RM-T**).
- Add $x \mu\text{L}$ of the appropriate **PC** component to the last two tubes (each containing a different reaction mixture) (**PC-C** to the tube containing **RM-C**; **PC-T** to **RM-T**).
- Close the reaction tubes and centrifuge them briefly.
- Place the reaction tubes into the reaction device and run the amplification program according to the following PCR profile.

Step	Temperature	Time	Data Collection	Cycles
Initial denaturation	95°C	5 min		1
Denaturation	95°C	10 s		} 35
Amplification	58°C	25 s	FAM and HEX	

NOTE 6: It is recommended that you prepare the Real Time PCR reaction in the laminar chamber to reduce the risk of reaction mixture contamination.

ANALYSIS EVALUATION

Step I: Analysis of reactions with PC components (positive controls)

Analyse each of the two reactions with the **PC** component (positive controls) to check the proper course of the Real Time PCR reaction.

	FAM	HEX	Result
1	+	-	Valid
2	+	+	Invalid: human DNA contamination
3	-	-	Invalid: no Real Time PCR reaction

+ means the PCR product amplification recorded as a fluorescence increase
- means no PCR product amplification and no fluorescence increase

If you obtain valid results for both control reactions, go to Step II of the analysis.

Step II: Control of the addition of DNA sample to the reaction

Analyse each of the other reactions for the addition of human genomic DNA to the Real Time PCR reaction.

	HEX	Result
1	+	Valid: DNA was added to the reaction
2	-	Invalid: no DNA was added to the reaction or inhibitors are present in the sample

+ means the PCR product amplification recorded as a fluorescence increase
- means no PCR product amplification and no fluorescence increase

If you obtain valid results for the DNA sample, you can proceed to the Step III of the analysis.

Step III: Determination of the *MTHFR* gene version in the samples

Check the Cq values for **RM-C** and **RM-T** components on the FAM channel. Combine them together to determine the version of the *MTHFR* gene. The alleles are heterozygous if the Cq values for the **RM-C** and **RM-T** reactions are comparable ($\Delta Cq < 2$). The alleles are homozygous if the Cq values for **RM-T** and **RM-C** reactions significantly differ from each other ($\Delta Cq > 5$). If $2 < \Delta Cq < 5$, reaction should be repeated.

Component		Cq values	Genotype
RM-C	RM-T		
+	-		Homozygotes (CC)
-	+		Homozygotes (TT)
+	+	$Cq_{RM-C} \approx Cq_{RM-T}$	Heterozygotes (CT)
+	+	$Cq_{RM-C} - Cq_{RM-T} > 5$	Homozygotes (TT)
+	+	$Cq_{RM-T} - Cq_{RM-C} > 5$	Homozygotes (CC)

+ means the PCR product amplification recorded as a fluorescence increase
- means no PCR product amplification and no fluorescence increase

ADDITIONAL REMARKS

- The TaqMan® probes have dark quenchers that do not generate additional fluorescent signals. For devices which require it (e.g. RotorGene 6000), select the **None** option as the quencher type.
- The Real Time PCR device should be calibrated (color compensation should be carried out) for fluorescent dyes.
- The total volume of the reaction mixture is 20 µL. Change of this volume may have a significant impact on some test parameters, including the detection limit.

WARNINGS AND PRECAUTIONS

- Take special care during DNA extraction. All samples should be treated as **potentially infectious material**.
- DNA extraction and the analysis of the rs1801133 polymorphism located in the *MTHFR* gene should be done by properly trained personnel in a professional laboratory which is allowed to work with potentially infectious material.
- Special attention should be paid to prevent cross-contamination of the DNA preparations during DNA extraction.
- To minimise false results, adhere to the following rules:
 - If possible, designate the separate stands for DNA extraction as well as preparation and performance of the Real Time PCR reaction;
 - Change protective clothing and gloves between the stages of DNA extraction and preparation of the Real Time PCR reaction;
 - Bench surfaces should be washed with DNA removing/destructive agents;
 - Use only sterile filter tips for automatic pipettes;
 - The **PC** components should be added to the reaction mixtures as the **last ones**. Before adding them, if possible, close the tubes with the DNA preparations;
- Do not eat, drink or smoke while using the kit;
- Wash your hands immediately after work with the kit;
- After contact of the skin or eyes with the kit components, gently wash them with water;
- It is recommended that you dispose of the kit after its expiry date or of any unused kit components as a laboratory waste.

TROUBLESHOOTING

Real Time PCR reaction

Problem	Problem		Possible cause	Suggestions
	FAM	HEX		
Incorrect fluorescence reading for positive control	+	+	Human genomic DNA contamination.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the contamination of the RM component with human genomic DNA.
	-	-	No PC component added or the RM component is of poor quality.	Repeat the Real Time PCR reaction for the positive control. If you receive an incorrect result again, assume the poor quality of the RM component (improper storage, improper transport conditions or exceeding the expiry date).
Incorrect fluorescence reading for the sample	-	-	No DNA preparation added to the reaction. Low DNA extraction yield and/or Real Time PCR inhibitors present. Too high DNA concentration. Poor quality of the RM component.	Measure the concentration of the DNA preparation and, if necessary, repeat the reaction with less DNA added to the reaction. In the absence of amplification in the HEX channel for DNA preparations and in the FAM channel for the positive control, assume the poor quality of the RM component. In case of amplification observed in the FAM channel for positive control and no amplification in the HEX channel for DNA preparations, assume contamination of the DNA preparation with PCR inhibitors or low efficiency of nucleic acid extraction.
	+	+	Contamination of the RM component.	Prepare a reaction with 11 µL of the RM component and 9 µL of water. No amplification in the FAM and HEX channels indicates no contamination. Amplification in one or both channels indicates contamination of the RM component with positive control, human genomic DNA and/or PCR product resulting from previous reactions.

CUSTOMER SERVICE

- Any problems or irregularities that arise during the use of the diagnostic kit can be reported by phone or email.
- Orders for the **AmpliSNiP (qPCR)** kits can be sent by email.

CONTACT

Support and customer care

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