MDR1 DELETION SNP TEST KIT USER GUIDE

CAT NO.: YSL-SNP-MDR1-100

100 reactions
with Lyophilised MasterMix

VERSION 5.7

For research use only

YouSeq Ltd
8 Moorside Place
Moorside Road
Winchester
SO23 7FX
United Kingdom

+44 333 577 6697
hello@youseq.com

youseq.com

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INTENDED USE

This YouSeq SNP detection kit employs competitive hydrolysis probe to determine the genotype of samples. The Wild Type sequences are detected in the FAM channel and the MDR1 deletion mutation (MDR1) is detected in the HEX Channel.

KIT CONTENTS

	Cap Colour	Volume
MDR1 specific primer/probe Wild Type (WT) probe (FAM Probe) Mutant (MUT) probe (HEX Probe)		110 µl
WT MDR1 positive control template		500 µl*
MUT MDR1 positive control template		500 µl*
Lyophilised Tetra™ 2X qPCR MasterMix		1.1 ml*
MasterMix resuspension buffer		1.5 ml
DNase/RNase free water		1.5 ml
Template resuspension buffer		1.5 ml
ROX passive reference dye		10 µl

* Supplied lyophilised and requires resuspension before use, see resuspension step below for instructions

RESUSPENSION

Resuspend the designated kit contents with the correct reagents as per the table below. Spin or gently tap the vials to ensure all the contents is at the bottom before opening.

After adding the resuspension reagent, pulse vortex the vials to ensure each is mixed well.

	Reagent	Volume
Lyophilised Tetra™ 2X qPCR MasterMix	MasterMix resus. buffer	1.1 ml
WT MDR1 positive control template	Template resus. buffer	500 µl
MUT MDR1 positive control template	Template resus. buffer	500 µl

MATERIALS REQUIRED BUT NOT PROVIDED

DNA Extraction kit – This qPCR test kit will work well with high quality DNA derived from any extraction kit with minimal PCR inhibitors present.

qPCR instrument with minimum 2 colour detection (FAM and VIC/HEX) that is capable of allelic discrimination.

Pipettes, micro centrifuge tubes and general laboratory equipment.

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ROX (PLATFORM DEPENDENT)

ROX is required for platforms that use ROX as a passive reference. The table below outlines the hardware platforms that require the addition of ROX.

If ROX is required, dilute the ROX supplied according to the table below, then add 5µl to the fully resuspended Tetra MasterMix.

	Instruments	Step 1: Volume of water to add to ROX tube	Step 2: Add to MasterMix vial
High ROX Instruments:	Applied Biosystems 7700, 7000, 7900, 7300, StepOne, StepOne Plus, and Roche capillary Lightcyclers 2.0	No Dilution Required	5 µl
Mid ROX Instruments:	Stratagene MX	75 µl	5 µl
Low ROX Instruments:	Applied Biosystems 7500 Platform, ViiA7 platforms, Quantstudio	130 µl	5 µl
All Other Machines		Not Required	Not Required

qPCR BENCH SIDE PROTOCOL

Clean and decontaminate all work surfaces, pipettes and other equipment prior to use to remove potentially contaminating nucleic acids.

REACTION SET UP

In a relevant sized sterile container (e.g., Microcentrifuge tube or Falcon tube), combine the required volume of each component, following the table below, to create enough Reaction Mix to cover all the required wells. Mix the combined reagents by briefly vortexing or inverting.

Component	Volume
Tetra™ 2X qPCR MasterMix	10 µl
Target specific primer/probe	1 µl
DNase/RNase free water	4 µl
Final Volume	15 µl

Then dispense 15 µl of the Reaction Mix into all the required wells and add 5 µl of sample into the relevant wells:

Component	Volume (per well)
Reaction Mix	15 µl
Extracted Sample Nucleic acid	5 µl
Final Reaction Volume	20 µl

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NEGATIVE CONTROL

For a negative control reaction, repeat the reaction set up above replacing the sample DNA with DNase/RNase free water.

Please note: Make sure to seal the sample and negative control wells before proceeding to the positive control step.

POSITIVE CONTROL STANDARDS

For homozygous Wild Type (WT) or homozygous Mutant (MUT) positive control reaction, repeat the reaction set up above but replace the sample DNA with 5 µL of the appropriate positive control template supplied with the kit.

Please note: Positive Control Template is a contamination risk and should be handled in a designated post-PCR environment.

qPCR AMPLIFICATION PROTOCOL

Prior to genotyping run, please confirm that the qPCR machine is capable of allelic discrimination. This YouSeq kit will work with any qPCR instrument capable of detecting FAM and VIC/HEX. Ensure your instrument has genotyping selected.

Run the following PCR protocol:

	Temperature	Time
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds

*Make sure to collect fluorogenic data through both the FAM and VIC/HEX channels during this step

For some machines a pre and post read is required for accurate genotyping. If this is the case, please run the following PCR protocol:

	Temperature	Time
Pre Read	60°C	30 seconds
Hot Start	95°C	3 minutes
45 cycles	95°C	15 seconds
	60°C*	60 seconds
Post Read	60°C	30 seconds

*Make sure to collect fluorogenic data through both the FAM and VIC/HEX channels during this step

INTERPRETATION OF RESULTS

Prior to genotyping analysis, ensure that all wells have suitable baselines and threshold applied, as this can dramatically impact genotyping result. Thresholds should be placed at 10% of the total EPF of the relevant PCT.
Should there be some low EPF amplification of the alternative allele in your PCT wells at a later Cq than the expected target, this is due to the competitive nature of the hydrolysis probes and can be considered background noise.

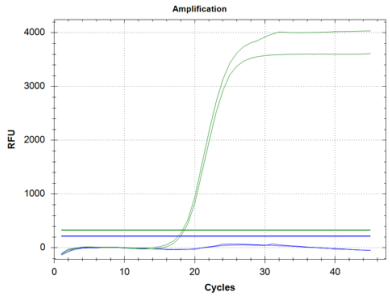
Positive control

Firstly, check the positive control performance. The positive control templates should amplify in a Cq range of approximately 18.5 +/- 2.
The test is valid if the following conditions are met:

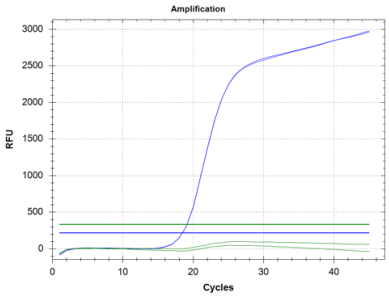
	FAM Channel	HEX Channel
Wild Type Positive Control	+	-*
Mutant Positive Control	-*	+
Negative Control	-	-

*There may be some low-level fluorescence through the other channel at a later Cq value

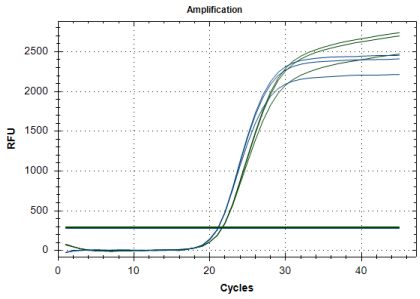
Wild Type



Mutant

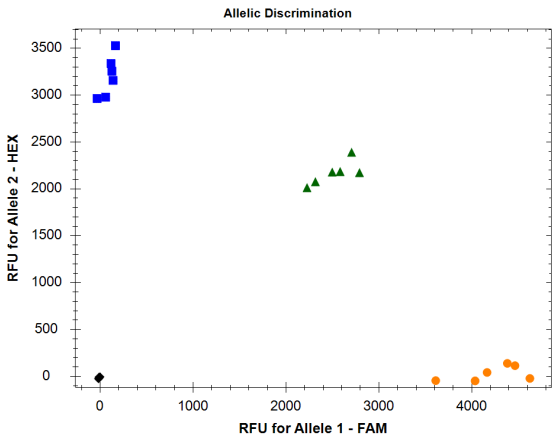


Heterozygous



Alleles should be clearly discriminated between 3 templates:

Allelic discrimination



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INTERPRETATION OF RESULTS CONTINUED

Negative control

In ideal circumstances, the negative control should deliver a flat line – negative result. However, it is not uncommon for background laboratory contamination to cause a very late signal.

Positive samples

Samples that are positive for the WT allele will deliver a defined "sigmoidal" amplification plot through the FAM channel.

Samples that are positive for the MUT allele will deliver a defined "sigmoidal" amplification plot through the HEX channel.

Samples that are positive for both the WT and MUT alleles will deliver a defined "sigmoidal" amplification plot through both the FAM & HEX channels at very similar Cq values.

Results interpretation at a glance:

	qPCR Signal			
WT primer/probe (FAM)	+	-*	+	-
MUT primer/probe (HEX)	-*	+	+	-
Result	**WT Positive result** Only the WT allele is present in the sample	**MUT Positive result** Only the MUT allele is present in the sample DNA	**Heterozygous Positive result** Both the WT & MUT alleles are present in the sample DNA	**Negative Result** Neither WT nor MUT alleles are present in the sample DNA

* There may be some low-level fluorescence through the other channel at a later Cq value.

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PRODUCT SPECIFICATIONS

Storing your kit

Store at -20°C from arrival. The qPCR kits shelf life is outlined as an expiry date on the pouch label.
Once you have prepared the positive control it can be stored frozen.

Use good quality DNA

Poor quality input nucleic acid is the biggest cause of test failure. The kit will work well with any source of good quality DNA. Good quality is defined as DNA with high integrity (not degraded) and with low levels of inhibitors present.

qPCR Machine Compatibility

Prior to genotyping analysis, please confirm that the qPCR machine is capable of allelic discrimination and all wells have suitable baselines and thresholds applied, as this can dramatically impact genotyping results.

Regulatory status

This product has been developed for Research Use Only and is not intended for diagnostic use. It should not be used for diagnosis of disease unless specifically approved by the regulatory authorities in the country of use.

Quality Control

In accordance with the YouSeq Ltd ISO EN 13485-certified Quality Management System, each lot of MDR1 deletion SNP Test Kit is tested against predetermined specifications to ensure consistent product quality. Design of the kit met our robust bioinformatic analysis requirements resulting in a clinically relevant detection profile based on available sequence information. The kit is periodically checked against newly available sequence information to remain clinically relevant.

Technical Assistance

For customer support, please contact:

e-mail: support@youseq.com
phone: +44 (0)333 577 6697

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