



PROTHROMBIN SNP TEST KIT USER GUIDE

CAT NO.: YS-SNP-Prothrom-100

100 reactions
with Frozen MasterMix

VERSION 3.3

For research use only



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INTENDED USE

This YouSeq SNP detection kit employs competitive hydrolysis probe to determine the genotype of samples. The Wildtype sequences are detected in the FAM channel and the Prothrombin mutation (rs1799963) is detected in the HEX Channel.

KIT CONTENTS

	Cap Colour	Volume
Prothrombin specific primer/probe WT probe (FAM Probe) Mutant probe (HEX Probe)		110 µl
WT Prothrombin positive control template		500 µl*
Mutant Prothrombin positive control template		500 µl*
Tetra 2X qPCR MasterMix		1 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 vial to ensure all the contents is at the bottom before opening.

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

	Reagent	Volume
WT Prothrombin positive control template	Template resus. buffer	500 µl
Mutant Prothrombin 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).

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 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

Combine the following reagents to create a final test reaction:

Component	Volume
Tetra 2X qPCR MasterMix	10 µl
Target specific primer/probe	1 µl
Extracted Sample DNA (recommended input amount of 10ng/rxn)	5 µl
DNase/RNase free water	4 µl
Final Volume	20 µl

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.



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POSITIVE CONTROL STANDARDS

For a homozygous WT or mutant MT 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.

qPCR AMPLIFICATION PROTOCOL

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

INTERPRETATION OF RESULTS

When analysing Sample Cq values, YouSeq recommends checking the threshold within the run file before interpreting the data. We would suggest setting the baseline threshold to 10% of the relevant positive control End Point Fluorescence (EPF).

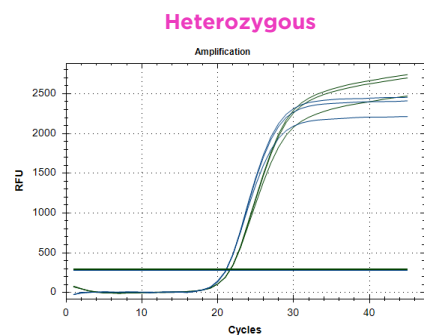
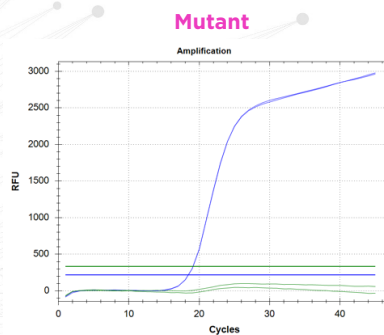
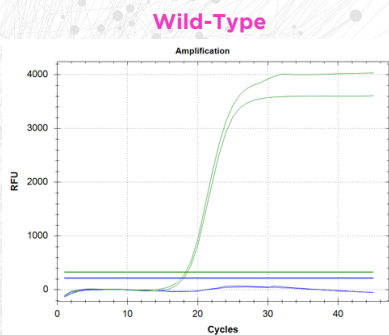
Positive control

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:

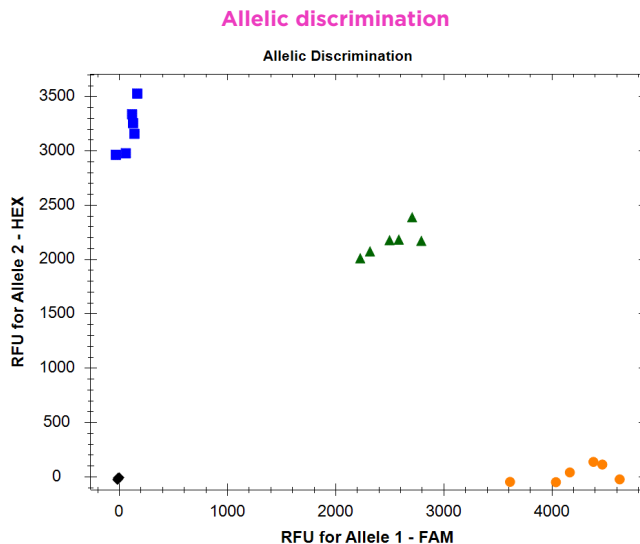
	FAM Channel	HEX Channel
WT Positive Control	+	-*
Mutant Positive Control	-	+
Negative Control	-	-

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



INTERPRETATION OF RESULTS CONTINUED

Alleles should be clearly discriminated between 3 templates:



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

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

Samples that are positive for both the WT and Mutant 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)	+*	-	+	-
Mutant primer/probe (HEX)	-	+	+	-
Result	WT Positive result Only the WT allele is present in the sample	Mutant Positive result Only the Mutant allele is present in the sample DNA	Heterozygous Positive result Both the WT & Mutant alleles are present in the sample DNA	Negative Result Neither WT nor Mutant alleles are present in the sample DNA

* There may be some low-level fluorescence through the HEX 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.

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 Prothrombin SNP Detection Kit is tested against predetermined specifications to ensure consistent product quality.

Technical Assistance

For customer support, please contact:

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