HELINI
BRCA1 & BRCA2
Real-time PCR Kit

Gene	Mutations
BRCA1	185delAG / 5382insC
BRCA2	6174delT

Instructions for use

For use with: Agilent, Bio-Rad, Roche Lightcycler-96, Roche-Z480/Cobas-480, Applied Bio systems [ABI], Thermo-Piko-Real, Rotor gene 5/6plex, Alta-96, Cepheid Real time PCR machines.

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Intended Use

The HELINI BRCA1 & BRCA2 Mutations Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of Major dominant three somatic mutations in the BRCA1 & BRCA2 genes associated with high risk of breast and/or ovarian cancer.

Kit components

Components	Volume Per reaction	No of vials	Volume Per vial
Probe PCR Master Mix	10µl	3	250µl
185delAG PP Mix	5µl	1	125µl
5382insC PP Mix	5µl	1	125µl
6174delT PP Mix	5µl	1	125µl
Positive control Mix	10µl	1	150µl
Water, PCR grade		1	4ml

Storage

- The kit is shipped on gel ice. Upon arrival, all components should be stored in -20°C. They are stable until the expiration date stated on the label.
- Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
- If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at 2 to 8°C should not exceed a period of 5 hours.

Material and instruments required

- Real-time PCR instrument having FAM channel
- Automatic Nucleic acid extraction system or spin column based purification kit for the purification of nucleic acids
- Desktop centrifuge having 13000rpm or above with a rotor for 1.5/2 ml reaction tubes
- Centrifuge with a rotor for PCR strips/tubes and 96 well plates
- Optical cap qPCR tubes or strips or 96 wells
- Micro Pipettes (variables)
- Micro Pipette tips with filters (disposable)
- Powder-free gloves (disposable)

[Please ensure that all instruments used have been installed, calibrated, checked and maintained according to the manufacturer´s instructions and recommendations.]

Product Use Limitations

- All reagents may exclusively be used in molecular diagnosis.
- The product is to be used by personnel specially instructed and trained in Molecular diagnosis.
- Strict compliance with the user manual is required for optimal PCR results.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens and kit components.
- Avoid microbial and nuclease (RNAse/RNase) contamination of the specimens and the components of the kit.
- Always use RNAse/RNase-free disposable pipette tips with aerosol barriers.
- Use separated and segregated working areas for sample preparation, reaction setup and amplification/detection activities.
- The workflow in the laboratory should proceed in unidirectional manner. Always wear disposable gloves in each area and change them before entering a different area.
- Store positive and/or potentially positive material separated from all other components of the kit.
- Do not open the reaction tubes/plates post amplification, to avoid contamination with amplicons.

- Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations.
- Do not autoclave reaction tubes after the PCR, since this will not degrade the amplified nucleic acid and will bear the risk to contaminate the laboratory area.
- Discard sample and assay waste according to your local safety regulations.

Technical Assistance

For technical assistance and more information, please contact;

0091-44-244490433
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Product description

HELINI BRCA1 & BRCA2 Real-time PCR Kit constitutes a ready-to-use system for the detection of BRCA1 & BRCA2 gene mutations using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification for the direct detection of the specific amplicon in fluorescence channels FAM. External positive control is supplied to assist the run.

Specificity

BRCA1 & BRCA2 mutation primer and probe have been designed for the specific and exclusive *in vitro* detection of BRCA1 & BRCA2 mutations.

Gene	Mutations
BRCA1	185delAG / 5382insC
BRCA2	6174delT

The primers and probe sequences in this kit have 100% homology with clinically relevant reference sequences based on a comprehensive bioinformatics analysis.

Dynamic linear range

The linear range was evaluated by analyzing a logarithmic dilution series of DNA concentrations ranging from 100ng/µl to 10ng/µl. At least six replicates per dilution were analyzed. The slopes are in expected limit in the recommended DNA concentration of 10ng/µl.

Analytical Sensitivity

The analytical sensitivity is defined as the concentration of DNA molecules (ng/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified mutation specific DNA from 1ng/µl to 100ng/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 2ng/µl

Note:

DNA Purification

Purified DNA is the starting material for the Real-time PCR assay. The quality of the purified DNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for DNA purification is compatible with real-time PCR technology.

If you are using a spin column based sample preparation procedure having washing buffers containing ethanol, it is highly recommended to perform an additional centrifugation step for 5 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

Detection Protocol

Things to do before starting

- Before use, all kit components need to be thawed completely, mixed by gently inverting and centrifuged briefly.
- Make sure that Positive and Negative control is included in every run.
- Include 0.5 reaction volume for pipetting error while calculating the volume for total number of reactions.

Components	**185**	**5382**	**6174**
Probe PCR Master Mix	10µl	10µl	10µl
PP Mix	5µl	5µl	5µl
DNA [>5ng/µl]	10µl	10µl	10µl
Final rxns volume	25µl	25µl	25µl

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Positive Control setup

Add 10µl of the Positive control

Centrifuge PCR vials briefly before placing into thermal cycler. *[Note: There should not be any bubbles in the reaction mix. Bubbles interfere with fluorescence detection.]*

Programming Thermal cycler

Sample volume	25µl
Fluorescence Dyes	FAM & HEX
Passive reference	None
Ramping rate	Default

Thermal Profile

	Step	**Time**	**Temp**
	Taq enzyme activation / Hold	15min	95ºC
35 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	63ºC
	Extension	20sec	72ºC

Data collection/Acquisition	**Targets**
FAM - **Wild**	185delAG / 5382insC / 6174delT
HEX - **Mutant**	185delAG / 5382insC / 6174delT

Reading the graph:

Step-1 –Negative and Positive control validation

Select the NTC and Positive control and view the graph of amplification.

The NTC must be flat with no Ct value. If required adjust the threshold value just above the NTC. The Positive control must be amplified.

NTC justifies NO contamination in the reagent as well as fine pipetting and its environment. PC justifies the reagents storage conditions and reaction parameters are as prescribed.

Step-2 Test Sample status

In FAM channel, select test sample well one by one, analyze the graph/amplification.

In HEX channel, select test sample well one by one, analyze the graph/amplification.

Qualitative interpretation of results:

Gene	Mutations	NTC	Positive control	Sample Amplified		Results
				FAM	HEX	
BRCA1	185delAG	Negative	Positive	Y	N	Homozygous Wild No deletion
BRCA1	185delAG	Negative	Positive	Y	Y	Heterozygous Wild/Mutant
BRCA1	185delAG	Negative	Positive	N	Y	Homozygous Mutant Deletion
BRCA1	5382insC	Negative	Positive	Y	N	Homozygous Wild No Insertion
BRCA1	5382insC	Negative	Positive	Y	Y	Heterozygous Wild/Mutant
BRCA1	5382insC	Negative	Positive	N	Y	Homozygous Mutant Insertion

Gene	Mutations	NTC	Positive control	Sample Amplified		Results
				FAM	HEX	
BRCA2	6174delT	Negative	Positive	Y	N	Homozygous Wild No deletion
BRCA2	6174delT	Negative	Positive	Y	Y	Heterozygous Wild/Mutant
BRCA2	6174delT	Negative	Positive	N	Y	Homozygous Mutant Deletion

Limitations

Good laboratory practice is essential for proper performance of this assay. Strict compliance with the instructions for use is required for optimal results.

Analysts should be trained and familiar with testing procedures and interpretation of results prior to performing the assay.

A false negative result may occur due to improper collection, transport or handling. Appropriate specimen collection, transport, storage and processing procedures are required for the optimal performance of this test.

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Extreme care should be taken to preserve the purity of the components of the kit and reaction setups. All reagents should be closely monitored for impurity and contamination. Any suspicious reagents should be discarded.

The presence of PCR inhibitors may cause under quantification, false negative or invalid results.

Potential mutations within the target regions of the genome covered by the primers and/or probes used in the kit may result in under quantification and/or failure to detect.

As with any diagnostic test, the HELINI BRCA1 & BRCA2 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

Quality Control

In accordance with the HELINI Biomolecules in house Quality Management System, each lot of HELINI BRCA1 & BRCA2 Real-time PCR kit is tested against predetermined specifications to ensure consistent product quality.

Explanations of symbols

In vitro diagnostic medical device

Catalogue number

Pack size – number of tests

Manufacturer

Manufactured by

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