HELINI
BCR-ABL
Real-time PCR Kit

[Quantitative detection of major (p210), minor (p190), and micro [p230] breakpoint transcripts]

Instructions for use

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

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Intended Use

The HELINI BCR-ABL Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of BCR-ABL fusion gene transcripts specific RNA.

Kit components

Components	Volume Per reaction	Number of vials	Volume Per vials
One step RT-PCR Master Mix	8µl	3	200µl
RT-Enzyme mix	2µl	3	50µl
Major-p210 PP Mix [Major PP Mix]	5µl	1	125µl
Minor-p190/Endo PP Mix [Minor/Endo PP Mix]	5µl	1	125µl
Micro-p230 PP Mix [Micro PP Mix]	5µl	1	125µl
BCR-ABL Positive control Mix [QS1]	10µl	1	250µl
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 & HEX channels
- 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;

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Product description

HELINI BCR-ABL Real-time PCR Kit constitutes a ready-to-use system for the detection of BCR-ABL fusion gene transcripts 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. In addition, it contains an endogenous control amplification system amplifies human ABL gene which identify possible PCR inhibition and RNA purification. External mutation positive control is supplied to assist the run.

Specificity

BCR-ABL mutation primer and probe have been designed for the specific and exclusive *in vitro* detection of BCR-ABL [Quantitative detection of Major (p210) - [e14a2 & e13a2], Minor (p190)-[e1a2], Micro p230-[e19a2] breakpoint gene fusion transcripts. 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 transcript specific pDNA concentrations ranging from 1.00E+09 to 1.00E+00copies/µl. At least six replicates per dilution were analyzed. The linear range is 1.00E+09 to 1.00E+00copies/µl.

Analytical Sensitivity

The analytical sensitivity is defined as the concentration of plasmid DNA/cDNA molecules (copies/µl) that can be detected with a positivity rate of 95%. The analytical sensitivity was determined by analysis of dilution series of quantified BCR-ABL specific cDNA/pDNA from 0.001copies to 10copies/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 0.35 copies per micro liter.

Note:

RNA Purification

- Whole blood samples should be anti-coagulated with potassium EDTA (K2-EDTA) and stored at 2–8°C for no more than 4 days before RNA extraction. Do not use frozen blood.

- Whole blood samples and RNA samples must be shipped under the same conditions as storage to avoid temperature changes during storage and shipment.

- Total RNA should be purified from 5 to 10 ml of peripheral whole blood collected in EDTA.

- Following isolation, purified RNA may be stored at –15 to –30°C or lower (–65 to –90°C) if long-term storage is required.

- The RNA concentration in the eluate should be in the range 70–200 ng/µl.

- If RNA concentration in the eluate is above the upper range limit, it should be adjusted to 200ng/µl with RNase-free water.

- Assay quality is largely dependent on the quality of input RNA. We recommend analyzing purified RNA by agarose† gel electrophoresis or spectrophotometer prior to analysis. [An A260/A280 ratio between 1.8 and 2.1 is indicative of highly purified RNA]

- Purified RNA is the starting material for the Real-time PCR assay. The quality of the purified RNA has a profound impact on the performance of the entire test system. It has to be ensured that the purification system used for RNA 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 10 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the RNA.

BCR-ABL - standard curve dilution series:

1. Pipette 90µl of Nuclease free water into three 1.5ml micro centrifuge tubes and label QS2 to QS4.
2. Pipette 10µl of BCR-ABL QS1 Positive control mix into tube QS2.
3. Vortex thoroughly and spin down briefly.
4. Change pipette tip and pipette 10µl from tube QS2 into tube QS3.
5. Vortex thoroughly and spin down briefly.
6. Repeat steps 4 and 5 to complete the dilution series.
7. Use 10µl per reaction.

Standard curve	**Copies/µl**
BCR-ABL QS-1	1000000
BCR-ABL QS-2	100000
BCR-ABL QS-3	10000
BCR-ABL QS-4	1000

Prepare every time freshly.

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	**BCR-ABL [Major]**	**BCR-ABL [Minor]**	**BCR-ABL [Micro]**
One Step RT-PCR Master Mix	8µl	8µl	8µl
RT-Taq Enzyme Mix	2µl	2µl	2µl
Primer Probe Mix	5µl	5µl	5µl
RNA	**10µl**	**10µl**	**10µl**
Final reaction volume	25µl	25µl	25µl

Negative Control setup [NTC]
Add 10µl of PCR grade water.

Positive control – Quantitative
Add 10µl of QS1 to QS4 [Use Major Primer Probe Mix for Standards]

Positive control – Qualitative
Add 10µl of QS1

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**
	Reverse transcriptase	20min	50ºC
	Taq enzyme activation / Hold	15min	95ºC
45 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec	60ºC
	Extension	20sec	72ºC

Data collection/ Acquisition	**Targets**
FAM	BCR-ABL-Major / Minor / Micro
HEX	Endogenous control

Reading the graph:

Step-1- Control gene

Select only test sample wells; view the graph of control gene amplification. A successful amplification Ct/Cq value must be between 16 to 32.

This range indicates that test sample is purified well and there is NO PCR inhibition in the reaction. Any sample value goes beyond Ct value 31 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Step-2- BCR-ABL

Select the NTC and Positive control, 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.

Select all four standards; analyze the amplification and its efficiency. [Slope and Ct value intervals]

Tests samples; note the copy numbers.

Qualitative interpretation:

Sample FAM	Negative control [FAM]	Positive control [FAM]	Control gene [HEX]	Interpretation
Positive	Negative	Positive	Positive	**BCR-ABL fusion gene RNA Detected**
Negative	Negative	Positive	Positive	**BCR-ABL Not Detected or beyond detection limit**
Negative	Negative	Negative	Negative	**Experiment fail**
Positive	Positive	Positive	Positive	**Experiment fail**

Note:
Poor quality of the RNA or problems during the qPCR steps result in low Endogenous control amplification Ct value. We recommend discarding results from samples giving endogenous control Ct value beyond 34.

Qualitative

Observation		Interpretation
FAM	**HEX**	
<37	<34	Assay Valid

Recommendation:

In FAM channel, the Ct value beyond 35 is required careful analysis. The analysis may include that the status of NTC amplification curve, threshold adjustment, linear/log scale view assessment, etc.,

Standard Curve analysis

- Interpret the values for unknown samples, only if the R>0.98
- Slope of calibrators is between-3.0 to -3.7
- PCR efficiency is between 85% and no amplification in FAM channel of negative control.

Calculating per ml

Input the machine indicated copy number into the following formula

$$\text{Result (Copies/ml)} = \frac{\text{Result (Copies/µl) x Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

Note:
Elution volume: must be typed in micro liter format, example 30µl, 60µl or 100µl. Sample volume: must be typed in milliliter format, example 0.2ml or 0.5ml

Observation	Interpretation
<30 Copies/ml	BCR-ABL RNA transcript detected, but below limit of detection
30 copies to 10^7 copies/mL	BCR-ABL RNA transcript detected and quantified
<1 x 10^7 copies/mL	BCR-ABL RNA transcript detected, but upper limit of quantitation

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 BCR-ABL 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 BCR-ABL 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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