HELINI
EGFR
Real-time PCR Kit

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.

CE IVD

REF 8506

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Intended Use

The HELINI EGFR Real-time PCR Kit is an in vitro nucleic acid amplification kit for the detection of 29 EGFR somatic mutations specific DNA in human genomic DNA.

Kit components

Components	Volume Per reaction	Number of vials	Volume Per vials
Probe PCR Master Mix	10µl	4	250µl
19 Deletions & Endogenous control Primer Probe Mix [19D & Endo PP Mix]	5µl	1	125µl
L858R & L861Q Primer Probe Mix [L858R & L816Q PP Mix]	5µl	1	125µl
T790M & S768I Primer Probe Mix [T790M & S768I PP Mix]	5µl	1	125µl
G719X & 3 Insertions Primer Probe Mix [G719 & 3Ins PP Mix]	5µl	1	125µl
Positive control mix	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 (DNase/RNase) contamination of the specimens and the components of the kit.
- Always use DNase/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
helinibiomolecules@gmail.com

Product description

HELINI EGFR Mutations Real-time PCR Kit constitutes a ready-to-use system for the detection of 29 somatic mutations in human EGFR genes using polymerase chain reaction (PCR). It contains reagents and enzymes for the specific amplification of the mutation specific region of the genome and for the direct detection of the specific amplicon in FAM & HEX channel. In addition, it contains an endogenous control amplification system to identify possible PCR inhibition and DNA purification efficiency.

Specificity

HELINI EGFRP Mutation Detection Real-time PCR kit use a property ARMS techniques based Pin-Tail™ HELINI Probe system. The Primer and Probe are high specific and for exclusive *in vitro* detection of all 29 mutations and have 100% homology with a broad range of 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 10ng/µl in triplicates. Under optimal PCR conditions, the analytical sensitivity is 1ng/µl genomic DNA.

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 3 min at approximately 17000 x g (~ 13000 rpm), using a new collection tube, prior to the elution of the DNA.

Endogenous Control template

Human gene amplification system is provided as endogenous control. A successful amplification of endogenous control in given range indicates that quality and quantity of the sample DNA, No PCR inhibitors and biological status of the test sample. The endogenous control is detected in HEX channel and gives a CT value of 23 +/- 10.

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	**T790M & S768I**	**L858R & L861Q**	**G716X & 3 Ins**	**19D & Endogenous Control**
Probe PCR Master Mix	10µl	10µl	10µl	10µl
Primer Probe Mix	5µl	5µl	5µl	5µl
Purified DNA	10µl	10µl	10µl	10µl
Final volume	25µl	25µl	25µl	25µl

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.]*

Negative Control setup [NTC]

Add 10µl of PCR grade water.

Qualitative Positive Control setup

Add 10µl of the Positive control.

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
38 cycles	Denaturation	20sec	95ºC
	Annealing/Data collection*	20sec**	60ºC
	Extension	20sec	72ºC

** Some qPCR machines may require minimum 30sec for data collection; in that case, set to 30sec, this will not affect the performance.

Data collection*

FAM channel	**HEX Channel**
T790M	Endogenous control
L858R	S768I
G719A/T/G	L861Q
19 Deletions	3 Insertions

Note: Mutation detection is based on the single nucleotide change, there is a chance of low background fluorescence amplification depends on the concentration of sample DNA. In order to rule out such issues, please set **threshold** as follows;
[Only if required]

Peltier based qPCR systems: [Chart - Y scale]
FAM: 100 or 1000
HEX: 50 or 500

Rotor-gene: [Chart - Y scale]
FAM: 0.2
HEX: 0.05

Reading the graph:

Step-1: Endogenous control validation:

Select wells having endogenous control, select HEX channel and view the graph of endogenous amplification. A successful amplification must be less than Ct 33. [Range 16 to 33].

This range indicates that test sample is collected and purified well and there is NO PCR inhibition in the reaction. Any sample value goes beyond Ct value 34 indicates that either sample has some issues in the purification or inhibiting PCR reaction.

Step-2

Select FAM, select and view one by one NTC. The NTC must be flat with no Ct value. If required adjust the threshold value as recommend. [Page-12]

Select and view one by one Positive control, it must be amplified.

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

Repeat the step 2 for HEX channel.

Step-3

Select FAM, Analyze the sample wells one by one and then select HEX; analyze the sample wells one by one. Note: Do not select all the wells at once for analysis.

Interpretation:

	FAM	**HEX**
Exon-20	T790M	S768I
Exon-21	L858R	L861Q
Exon-18	G716X	--
Exon-20		3 Insertions
Exon-19	19 deletions	--

Based on the amplification signal, report as follows;

"EGFR Exon-XXXX mutation detected"

Mutations:

Exon-18	Exon-19	Exon-20	Exon-21
G719A – 2156G>C G719S – 2155G>A G719C – 2155G>T	2235-2249 del 15 2235-2252>AAT del18 2236-2253 del 18 2237-2251 del 15 2237-2254 del 18 2237-2255>T del 19 2236-2250 del 15 2238-2255 del 18 2238-2248>GC del 11 2238-2252>GCA del 15 2239-2247 del 9 2239-2253 del 15 2239-2256 del 18 2239-2248>C del 10 2239-2258>CA del 20 2240-2251 del 12 2240-2257 del 18 2240-2254 del 15 2239-2251>C del 13	T790M – 2369C>T S768I – 2303G>T 2307-2308 ins GCCAGCGTG 2319-2320 ins CAC 2310-2311 ins GGT	L858R – 2573T>G L861Q – 2582T>A

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 if inadequate numbers of organisms are present in the sample 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 pathogen's 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 EGFR 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 EGFR 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

HELINI *Biomolecules,*
Ohmlina, 26, 2nd Avenue,
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