EGFR Real-time PCR Kit

(For Qualitative Detection)

REF 8506

IVD -15°C -25°C

INTENDED USE

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 CONTENTS

Components	No. of vials	Volume Per vials
Probe PCR Master Mix	4	250µl
19 Deletions & Endogenous control Primer Probe Mix [19D & Endo PP Mix]	1	125µl
L858R & L861Q Primer Probe Mix [L858R & L816Q PP Mix]	1	125µl
T790M & S768I Primer Probe Mix [T790M & S768I PP Mix]	1	125µl
G719X & 3 Insertions Primer Probe Mix [G719 & 3Ins PP Mix]	1	125µl
Positive control mix	1	250µl
Water, PCR grade		4ml

STORAGE

1. 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.
2. Repeated thawing and freezing should be avoided, as this might affect the performance of the assay.
3. 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.

PRODUCT DESCRIPTION

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.

PRODUCT DESCRIPTION

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.

SPECIFICITY

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.

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.

DNA PURIFICATION

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 21 +/- 10.

Detection Protocol

- 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

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

Cycles	Step	Time	Temp
1	Taq enzyme activation / Hold	15min	95ºC
45	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
G716A/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 32. [Range 16 to 31].

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.

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 –FAM - Test Sample status

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

	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

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.

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 HPV 6/11 Real-time PCR results need to be interpreted in consideration of all clinical and laboratory findings.

QUALITY CONTROL

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

Index of symbols

Consult instructions for use	LOT Batch code	Tests per kit
IVD For *in vitro* diagnostic use only	Do not re-use	Keep dry
8°C 2°C Temperature limit - 25 °C to - 15 °C	Date of manufacture	Biologial Risks
Manufacturer	Use-by date	

Marketed by:

Athenese-Dx Pvt. Ltd.
Module No. 407 & 408, 4th Floor,
TICEL Bio Park II, No. 5, CSIR Road.
Taramani, Chennai-600113, India
Tel: +91-44-22541131
E-mail: info@athenesedx.com
Website: www.athenesedx.com

PI-8506 Rev. A
Effective date: 05.08.2020
English version

Manufactured by:

HELINI Biomolecules,
Ohmlina, 26, 2nd Avenue,
Khuthubi Complex,Vettuvankeni,
Chennai 600 115.