

INTRODUCTION

Epidermal growth factor receptor (EGFR) is a transmembrane protein that plays a central role in multiple cellular processes including cell proliferation, survival and apoptosis. Overexpression and oncogenic mutations in EGFR have been associated in the pathogenesis and progression of different carcinoma types, notably in non-small cell lung cancer. A single C2615T mutation within exon 20 of EGFR results in a substitution of threonine for methionine at codon 790. Patients who develop this mutation acquire resistance to first line EGFR tyrosine kinase inhibitor therapy and as such, monitoring the T790M mutation status is crucial to guide treatment decisions.

KIT CONTENTS

The Clarity™ EGFR T790M Mutation Quantification Kit provides ready-to-use reagents for the detection and quantification of T790M mutation on the Clarity™ digital PCR system (Cat. No. 10001). Each kit includes reagents (Table 1) sufficient to perform 48 reactions.

Reagents Supplied	Volume (µL)
T790M Primer and Probe Mix	65
T790M dPCR Master Mix (2x)	415
PCR grade Water	200
T790M Positive Control (Allele frequency of 50%) [^]	45

[^]Sufficient for at least 12 reactions

STORAGE AND STABILITY

The Clarity™ EGFR T790M Mutation Quantification Kit should be stored at -20°C upon receipt. Avoid repeated freezing and thawing of kit contents. The kit is stable through the expiry date indicated on the kit label.

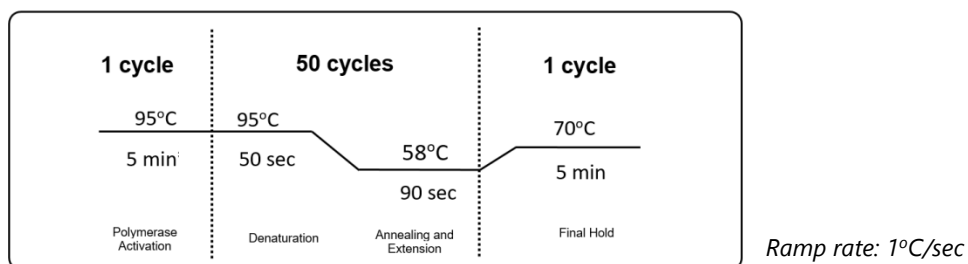
EXPERIMENTAL PROCEDURE

1. Thaw reagents at room temperature. When reagents are completely thawed, mix contents by gentle vortexing and centrifuge to collect contents at the bottom of the tubes.
2. Prepare each reaction mix according to the following:

No.	Reagents	Volume (µL)
1	JN Solution (20X)*	0.75
2	T790M Primer and Probe Mix	1.2
3	T790M dPCR Master Mix (2x)	7.5
4	DNA sample or control	3
5	PCR grade Water	2.55
Total vol		15

*Part of Clarity™ 10K consumables package (Cat. No. 10011). Not provided in this kit.

3. Mix thoroughly by pipetting up and down. Centrifuge to collect contents at the bottom of the tubes.
4. Load sample onto Clarity™ Tube-strips and perform sealing according to instructions provided in the Clarity™ Digital PCR System User Manual.
5. Perform PCR using a deep-well (0.2 ml) thermal cycler using the recommended conditions as shown.



4. Proceed with data acquisition and analysis on the following channels – T790M (FAM; default setting) and wild-type (HEX, -1 setting). Refer to the Clarity™ Digital PCR System User Manual for detailed data acquisition and analysis instruction.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.