

## INTRODUCTION

Epidermal growth factor receptor (EGFR) is a transmembrane protein which regulates signal transduction pathways involved in multiple cellular processes, such as cell proliferation, survival and apoptosis. EGFR overexpression and mutation have been implicated in the pathogenesis of several human malignancies, including non-small cell lung cancer (NSCLC). The T2573G mutation results in an amino acid substitution at position 858 in human EGFR, from a leucine (L) to an arginine (R). The L858R mutation accounts for the majority of EGFR-mutated NSCLC, and it displays higher sensitivity to first line EGFR tyrosine kinase inhibitor therapy as compared to wild type EGFR. Therefore, monitoring the L858R mutation status is crucial for the prescription of targeted therapies and prediction of their clinical responses.

## KIT CONTENTS

The Clarity™ EGFR L858R Mutation Quantification Kit provides ready-to-use reagents for the detection and quantification of L858R mutation on the Clarity™ digital PCR system (Cat. No. 10001). Each kit includes reagents sufficient to perform 48 reactions.

Reagents Supplied	Volume (µL)
L858R Primer and Probe Mix	33
L858R dPCR Master Mix (2X)	415
PCR grade Water	220
L858R Positive Control (Allele frequency of 50%) <sup>^</sup>	45

<sup>^</sup>Sufficient for at least 12 reactions

## STORAGE AND STABILITY

The Clarity™ EGFR L858R 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.

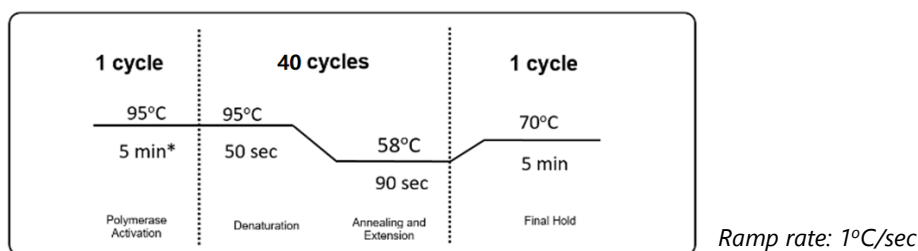
## EXPERIMENTAL PROCEDURE

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 ( $\mu\text{L}$ )
1	JN Solution (20X)*	0.75
2	L858R Primer and Probe Mix	0.6
3	L858R dPCR Master Mix (2X)	7.5
4	DNA sample or control	3
5	PCR grade Water	3.15
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 with default setting for both FAM and HEX channels. 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.*