

INTRODUCTION

Isocitrate dehydrogenase 2 (IDH2), a nicotinamide adenine dinucleotide phosphate (NADP)-dependent enzyme in mitochondria, is a critical component of the tricarboxylic acid cycle and involves in diverse cellular processes including histone demethylation and DNA modification. Two most frequently detected IDH2 mutations are G419A and G515A, which results in the R140Q and R172K amino acid substitutions respectively. These recurrent somatic mutations in the arginine residues promote tumorigenesis and cell proliferation, and have been identified in multiple malignancies such as acute myeloid leukemia, angioimmunoblastic lymphomas and glioma. Hence detection of the IDH2 R140Q and R172K mutations is clinically significant for tumour diagnosis, selection of targeted treatments and monitoring of disease prognosis.

KIT CONTENTS

The Clarity™ IDH2 Mutation Quantification Kit provides ready-to-use reagents for the detection and quantification of both IDH2 R140Q and R172K mutations on the Clarity™ digital PCR system (Cat. No. 10001). Each kit includes reagents sufficient to perform 48 reactions for R140Q and R172K detection.

Reagents Supplied	Volume (µL)
IDH2 R140Q Primer and Probe Mix	33
IDH2 R172K Primer and Probe Mix	33
IDH2 dPCR Master Mix (2X)	830
PCR grade Water	440
IDH2 R140Q Positive Control [^]	45
IDH2 R172K Positive Control [^]	45

[^]Sufficient for at least 12 reactions

STORAGE AND STABILITY

The Clarity™ IDH2 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.

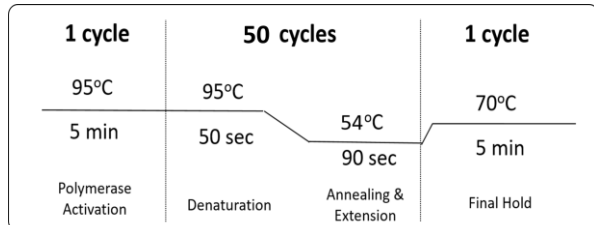
EXPERIMENTAL PROCEDURE

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	IDH2 Primer and Probe Mix (R140Q or R172K)	0.6
3	IDH2 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.



Ramp rate: 1°C/sec

4. Proceed with data acquisition and analysis with +2 setting for FAM channel and default setting for HEX channel. 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.