

# IDH1/2 Mutation Detection Kit

## Real-Time PCR assay

- Detects IDH1 R132, R100, IDH2 R140, and R172 mutations
- 38 tests per kit
- Available as RUO (US) / IVD (EU)

## INTENDED USE:

The IDH1/2 Mutation Detection Kit is intended for the detection of IDH1 and IDH2 somatic mutations (listed in Table 1) in genomic DNA isolated from human tissue. For research use only in the U.S. Not for use in diagnostic procedures. For in vitro diagnostic use in the European Union.

| Gene | Exon | AA Change | Nt Change          | HGVS Change                                 | HGVS nt Change                    | Cosmic ID  | Detected by Primer No. |
|------|------|-----------|--------------------|---------------------------------------------|-----------------------------------|------------|------------------------|
| IDH1 | 4    | R132H     | c.395G>A           | LRG_610p1.p.<br>(Arg132His)                 | LRG_610t1:c.395G>A                | COSM28746  | 1                      |
|      |      | R132C     | c.394C>T           | LRG_610p1.p.<br>(Arg132Cys)                 | LRG_610t1:c.394C>T                | COSM28747  |                        |
|      |      | R132S     | c.394C>A           | LRG_610p1.p.<br>(Arg132Ser)                 | LRG_610t1:c.394C>A                | COSM28748  |                        |
|      |      | R132G     | c.394C>G           | LRG_610p1.p.<br>(Arg132Gly)                 | LRG_610t1:c.394C>G                | COSM28749  |                        |
|      |      | R132L     | c.395G>T           | LRG_610p1.p.<br>(Arg132Leu)                 | LRG_610t1:c.395G>T                | COSM28750  |                        |
|      |      | R132P     | c.395G>C           | LRG_610p1.p.<br>(Arg132Pro)                 | LRG_610t1:c.395G>C                | COSM221574 |                        |
|      |      | R132V     | c.394_395<br>CG>GT | NM_005896.4(I<br>DH1J001):p.<br>(Arg132Val) | NM_005896.4:c.394_395<br>delinsGT | COSM28751  |                        |
|      |      | R100Q     | c.299G>A           | LRG_610p1.p.<br>(Arg100Gln)                 | LRG_610t1:c.299G>A                | COSM88208  | 2                      |

| Gene | Exon | AA Change | Nt Change | HGVS Change                                 | HGVS nt Change       | Cosmic ID   | Detected by Primer No. |
|------|------|-----------|-----------|---------------------------------------------|----------------------|-------------|------------------------|
| IDH2 | 4    | R172K     | c.515G>A  | LRG_611p2.p.<br>(Arg172Lys)                 | LRG_611t2:c.515G>A   | COSM33733   | 3                      |
|      |      | R172M     | c.515G>T  | LRG_611p2.p.<br>(Arg172Met)                 | LRG_611t2:c.515G>T   | COSM33732   |                        |
|      |      | R172W     | c.514A>T  | LRG_611p2.p.<br>(Arg172Trp)                 | LRG_611t2:c.514A>T   | COSM34039   |                        |
|      |      | R172G     | c.514A>G  | LRG_611p2.p.<br>(Arg172Gly)                 | LRG_611t2:c.514A>G   | COSM33731   |                        |
|      |      | R172S     | c.516G>T  | LRG_611p2.p.<br>(Arg172Ser)                 | LRG_611t2:c.516G>T   | COSM34090   |                        |
|      |      | R140Q     | c.419G>A  | NM_002168.4(I<br>DH2J001):p.<br>(Arg140Gln) | NM_002168.4:c.419G>A | COSM41590   | 4                      |
|      |      | R140W     | c.418C>T  | LRG_611p2.p.<br>(Arg140Trp)                 | LRG_611t2:c.418C>T   | COSM41877   |                        |
|      |      | R140L     | c.419G>T  | LRG_611p2.p.<br>(Arg140Leu)                 | LRG_611t2:c.419G>T   | COSM41875   | 5                      |
|      |      | R140G     | c.418C>G  | LRG_611p2.p.<br>(Arg140Gly)                 | LRG_611t2:c.418C>G   | COSM1737874 |                        |
|      |      |           |           |                                             |                      |             |                        |

## FOR USE WITH:

- Applied Biosystems QuantStudio™ 5 Fast, 7500 Fast & Standard Instruments
- BioRad CFX96
- Qiagen Rotor-Gene® Q
- Roche LightCycler® 480

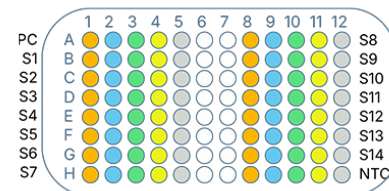
## PACKAGE CONTENTS

This product contains the following materials sufficient for 38 reactions per Primer Mix, including all controls and samples.

| Description                          | Tubes |
|--------------------------------------|-------|
| Mutation Detection Reaction Mix (2X) | 2     |
| IDH Primer Mix (1-5)                 | 5     |
| IDH Positive Control Mix             | 1     |

## PLATE SETUP

Suggested plate layout for a single experiment analyzing 14 unknown samples. Orange, blue, green, yellow, and grey represent wells containing Primer/Probe Mix 1-5, respectively.



## PRINCIPLE OF THE ASSAY

The IDH1/2 Mutation Detection Kit is a multiplex polymerase chain reaction (PCR)-based assay that uses allele-specific primers to identify the presence of IDH1 or IDH2 mutations in a total of 5 reactions per sample. The assay is designed to preferentially amplify mutant DNA even in samples that contain mostly wild-type DNA.

The amplification product is detected by fluorescent hydrolysis probes. Each probe contains a fluorophore, FAM or VIC. Probes complementary to the IDH1 and IDH2 genes are labeled with the FAM fluorophore, while the probe complementary to the endogenous control gene is tagged with the VIC fluorophore. Signal from the VIC tagged probe indicates that there is sufficient amplifiable DNA template in the reaction, while a signal from the FAM fluorophore indicates the presence of a mutation.

## HANDLING AND STORAGE

This product is shipped on frozen ice packs. The contents of the shipment should be stored at -25°C to -15°C non-frost-free freezer immediately upon receipt.

- Store all unopened components in their original containers.
- Primer/probe mixes should be protected from light at all times to prevent photo bleaching of the fluorescent dyes.
- Centrifuge the tubes before opening.
- The expiration date of each component is printed on each tube label. This product will maintain its performance through this date. Its performance is not guaranteed after the expiration date.

## EPAS DATA ANALYSIS

Instrument's settings must be saved to the instrument analysis file prior to importing into EPAS. For detailed instructions for use of EPAS download the EPAS IFU. EPAS may not be available for all instruments.

Due to cross-reactivity, some samples may have positive signals for multiple mutations. In such cases, the sample is positive only for the mutation that corresponds to the reaction with the most prominent signal (lowest Ct value). The cross-reactivity profile is described on page 2. Follow the naming conventions listed in the tables below for Successful IDH Interpretation in EPAS, these are case-sensitive:

| IDH1/2 Mutation | FAM     | VIC        |
|-----------------|---------|------------|
| R132            | R132    | R132_IC    |
| R100            | R100    | R100_IC    |
| R172            | R172    | R172_IC    |
| R140Q/W         | R140Q/W | R140Q/W_IC |
| R140L/G         | R140L/G | R140L/G_IC |

## ASSAY PERFORMANCE

### Limit of Blank

LoB of the assay was tested with 1 lot of nuclease-free water (No Template Control) and at least 5 wildtype (for IDH) FFPE sample using 3 distinct IDH-RT38 lots. FFPE samples were prepared at a concentration near the maximum limit of the reportable range for the assay (i.e., at a VIC Ct of ~27). At least four replicates of each FFPE sample and water lot were tested with each kit lot of 5 reactions of the IDH1/2 Mutation Analysis Kit on a QuantStudio™ 5 instrument.

| Reaction   | Channel | FFPE Samples (IC Ct of 27)             |       |                | Nuclease-Free Water |       |                |
|------------|---------|----------------------------------------|-------|----------------|---------------------|-------|----------------|
|            |         | Mean Ct                                | Ct SD | % Correct Call | Mean Ct             | Ct SD | % Correct Call |
| Reaction 1 | VIC     | 26.74                                  | 0.29  | N/A            | 40.0                | 0.0   | N/A            |
|            | FAM     | 40.0<br><small>(undetermined)</small>  | 0.02  | 100%           | 40.0                | 0.0   | 100%           |
| Reaction 2 | VIC     | 26.71                                  | 0.35  | N/A            | 40.0                | 0.0   | N/A            |
|            | FAM     | 40.0                                   | 0.00  | 100%           | 40.0                | 0.0   | 100%           |
| Reaction 3 | VIC     | 26.16                                  | 2.14  | N/A            | 40.0                | 0.0   | N/A            |
|            | FAM     | 39.83<br><small>(undetermined)</small> | 0.51  | 98%            | 40.0                | 0.0   | 100%           |
| Reaction 4 | VIC     | 26.54                                  | 0.37  | N/A            | 40.0                | 0.0   | N/A            |
|            | FAM     | 39.85<br><small>(undetermined)</small> | 0.39  | 95%            | 40.0                | 0.0   | 100%           |
| Reaction 5 | VIC     | 26.82                                  | 0.27  | N/A            | 40.0                | 0.0   | N/A            |
|            | FAM     | 40.0                                   | 0.00  | 100%           | 40.0                | 0.0   | 100%           |

Note: Undetermined Ct values have been assigned a value of 40 for statistical purposes.

### Limit of Detection

LoD of the assay was assessed with mutant positive gBlocks Gene Fragments-based controls (except for mutation R132H where Horizon Dx standard, HD677, was used) using 3 distinct IDH-RT38 lots. Wildtype genomic DNA prepared at concentrations near both limits of the reportable range for the assay (i.e., at a VIC Ct of ~27 and ~30) were utilized as background. For each mutation, sample preparations at different percent mutant were generated by adding specific copies of mutant gBlock to wildtype DNA at a VIC Ct of ~27 and ~30. The assay was performed using 2 sample preparations with 4 replicates per sample per kit lot (24 replicates per sample).

### LoD in samples tested on QuantStudio™ 5

| Reaction   | Mutation | At IC Ct of ~27 |          |          |        |        | Mean Ct values at IC Ct of ~30 |          |          |        |        |
|------------|----------|-----------------|----------|----------|--------|--------|--------------------------------|----------|----------|--------|--------|
|            |          | LoD             | Mean VIC | Mean FAM | SD VIC | SD FAM | LoD                            | Mean VIC | Mean FAM | SD VIC | SD FAM |
| Reaction 1 | R132H    | 15%             | 27.56    | 33.91    | 0.10   | 0.39   | 15%                            | 29.95    | 36.36    | 0.18   | 1.00   |
|            | R132C    | 10%             | 27.11    | 34.88    | 0.11   | 0.46   | 8%                             | 29.67    | 35.72    | 0.19   | 0.49   |
|            | R132S    | 5%              | 26.93    | 37.27    | 0.26   | 0.73   | 5%                             | 29.34    | 37.45    | 0.24   | 0.67   |
|            | R132G    | 5%              | 26.9     | 37.16    | 0.25   | 0.72   | 5%                             | 29.40    | 37.55    | 0.11   | 0.11   |
|            | R132L    | 10%             | 27.06    | 36.35    | 0.16   | 0.71   | 20%                            | 29.54    | 35.44    | 0.17   | 0.43   |
|            | R132P    | 5%              | 26.94    | 36.26    | 0.29   | 0.87   | 5%                             | 29.25    | 37.05    | 0.22   | 0.75   |
|            | R132V    | 5%              | 26.91    | 36.74    | 0.27   | 0.90   | 20%                            | 29.46    | 34.06    | 0.20   | 0.29   |
|            | R100Q    | 5%              | 26.67    | 35.45    | 0.24   | 0.71   | 5%                             | 29.16    | 35.75    | 0.19   | 0.71   |
|            | R172K    | 1%              | 27.87    | 36.42    | 0.14   | 0.43   |                                |          |          |        |        |
| Reaction 3 | R172M    | 2%              | 27.88    | 36.10    | 0.17   | 0.64   |                                |          |          |        |        |
|            | R172W    | 15%             | 27.98    | 36.32    | 0.32   | 0.53   |                                |          |          |        |        |
|            | R172S    | 5%              | 27.97    | 34.29    | 0.14   | 1.31   |                                |          |          |        |        |
|            | R172Q    | 20%             | 27.82    | 35.79    | 0.12   | 0.72   |                                |          |          |        |        |
| Reaction 4 | R140Q    | 15%             | 27.67    | 33.69    | 0.36   | 0.46   | 15%                            | 29.85    | 36.13    | 0.22   | 0.51   |
|            | R140W    | 5%              | 26.96    | 35.15    | 0.29   | 0.59   | 5%                             | 30.46    | 36.96    | 0.36   | 0.55   |
| Reaction 5 | R140L    | 5%              | 26.88    | 35.75    | 0.25   | 0.46   | 5%                             | 29.65    | 37.87    | 0.17   | 0.75   |
|            | R140G    | 5%              | 26.87    | 35.08    | 0.26   | 0.94   | 5%                             | 30.03    | 35.85    | 0.40   | 1.63   |

### LoD in samples tested on Bio-Rad CFX96

| Reaction   | Mutation | At IC Ct of ~27 |          |          |        |        | Mean Ct values at IC Ct of ~30 |          |          |        |        |
|------------|----------|-----------------|----------|----------|--------|--------|--------------------------------|----------|----------|--------|--------|
|            |          | LoD             | Mean VIC | Mean FAM | SD VIC | SD FAM | LoD                            | Mean VIC | Mean FAM | SD VIC | SD FAM |
| Reaction 1 | R132H    | 5%              | 27.45    | 35.93    | 0.10   | 0.78   | 5%                             | 29.68    | 38.10    | 0.11   | 0.69   |
|            | R132C    | 5%              | 26.87    | 35.51    | 0.09   | 0.67   | 2.5%                           | 29.52    | 35.21    | 0.11   | 0.45   |
|            | R132S    | 5%              | 27.35    | 33.71    | 0.08   | 0.38   | 5%                             | 29.52    | 35.59    | 0.10   | 0.40   |
|            | R132G    | 5%              | 27.35    | 33.51    | 0.09   | 0.28   | 5%                             | 29.66    | 36.07    | 0.13   | 0.57   |
|            | R132L    | 5%              | 26.82    | 36.00    | 0.11   | 0.71   | 2.5%                           | 29.60    | 35.67    | 0.08   | 0.37   |
|            | R132P    | 5%              | 27.00    | 36.27    | 0.51   | 1.08   | 2.5%                           | 29.62    | 35.69    | 0.15   | 0.55   |
|            | R132V    | 5%              | 26.90    | 35.78    | 0.10   | 0.63   | 2.5%                           | 29.69    | 35.56    | 0.11   | 0.49   |
| Reaction 2 | R100Q    | 5%              | 26.93    | 33.57    | 0.18   | 0.37   | 5%                             | 29.55    | 35.81    | 0.11   | 0.52   |
| Reaction 3 | R172K    |                 |          |          |        |        | 2%                             | 29.84    | 36.15    | 0.13   | 0.63   |
|            | R172M    |                 |          |          |        |        | 2%                             | 29.76    | 37.19    | 0.14   | 0.71   |
|            | R172W    |                 |          |          |        |        | 15%                            | 29.97    | 37.49    | 0.35   | 0.48   |
|            | R172S    |                 |          |          |        |        | 5%                             | 29.73    | 36.54    | 0.19   | 0.61   |
|            | R172Q    |                 |          |          |        |        | 30%                            | 29.69    | 36.00    | 0.18   | 0.86   |
| Reaction 4 | R140Q    | 10%             | 27.25    | 34.71    | 0.14   | 0.42   | 5%                             | 29.74    | 37.66    | 0.12   | 0.87   |
|            | R140W    | 5%              | 26.66    | 35.38    | 0.15   | 0.66   | 1%                             | 28.94    | 34.54    | 0.08   | 0.33   |
|            | R140L    | 5%              | 27.35    | 37.35    | 0.11   | 0.53   | 1%                             | 29.70    | 38.14    | 0.16   | 0.60   |
| Reaction 5 | R140G    | 5%              | 26.81    | 36.07    | 0.10   | 0.58   | 1%                             | 28.95    | 34.39    | 0.09   | 0.42   |

### Cross Reactivity

Dilutions of synthetic mutant DNA at an IC Ct of about 27 were made to yield 50% mutant allelic burden over wild-type background. The 50% samples were analyzed in duplicate using three different kit lots. The Ct values of each mutation was averaged between the different runs and presented in the table below. Blank Ct values indicate that the Ct value was undetermined.

### QuantStudio™ 5 instrument

| Reaction Mixes | 50% mutant, VIC Ct ~27                       |         |         |         |         |       |
|----------------|----------------------------------------------|---------|---------|---------|---------|-------|
|                | Mutations within each Primer/Probe Mix Group |         |         |         |         |       |
|                | Group 1                                      | Group 2 | Group 3 | Group 4 | Group 5 |       |
|                | Mix 1                                        | 28.63   |         |         |         |       |
|                | Mix 2                                        |         | 27.32   |         |         |       |
|                | Mix 3                                        | 37.81   | 38.34   | 29.30   | 38.57   | 35.17 |
|                | Mix 4                                        |         |         |         | 29.08   | 34.19 |
|                | Mix 5                                        |         |         |         | 39.22   | 28.53 |

Due to cross-reactivity, some samples may have positive signals for multiple mutations. In such cases, the sample is positive only for the mutation that corresponds to the reaction with the most prominent signal (lowest Ct value).  
The R140L/Q may show non-specific amplification in reactions 3 and 4 due to cross-reactivity

### Bio-Rad CFX96

| Reaction Mixes | 50% mutant, VIC Ct ~27                       |         |         |         |         |       |
|----------------|----------------------------------------------|---------|---------|---------|---------|-------|
|                | Mutations within each Primer/Probe Mix Group |         |         |         |         |       |
|                | Group 1                                      | Group 2 | Group 3 | Group 4 | Group 5 |       |
|                | Mix 1                                        | 28.02   |         |         |         |       |
|                | Mix 2                                        |         | 27.59   |         |         |       |
|                | Mix 3                                        | 39.14   | 38.34   | 26.85   | 39.35   | 37.24 |
|                | Mix 4                                        | 38.67   |         | 39.64   | 28.48   | 32.23 |
|                | Mix 5                                        |         |         |         |         | 27.48 |

### Precision

Precision of the assay was assessed using mutant positive gBlocks Gene Fragments-based controls. Mutant samples were prepared at 1-3x LoD in a background of wildtype genomic DNA. The assay was performed across 3 lots of IDH-RT38 for a total of 18-24 replicates per sample.

### QuantStudio™ 5 instrument

| Mutation | Mean FAM Ct | SD of FAM Ct | %CV of FAM Ct | Mean VIC Ct | SD VIC Ct | %CV of VIC Ct |
|----------|-------------|--------------|---------------|-------------|-----------|---------------|
| R132H    | 35.06       | 1.23         | 3.51%         | 29.37       | 0.30      | 1.01%         |
| R132C    | 34.40       | 1.02         | 2.96%         | 29.35       | 0.31      | 1.05%         |
| R132S    | 35.44       | 0.68         | 1.92%         | 29.65       | 0.14      | 0.48%         |
| R132G    | 35.50       | 0.52         | 1.47%         | 29.53       | 0.33      | 1.1%          |
| R132L    | 33.79       | 0.89         | 2.63%         | 29.41       | 0.35      | 1.18%         |
| R132P    | 35.16       | 0.65         | 1.86%         | 29.50       | 0.16      | 0.55%         |
| R132V    | 35.47       | 0.72         | 2.02%         | 29.27       | 0.26      | 0.88%         |
| R100Q    | 35.40       | 0.64         | 1.8%          | 29.58       | 0.45      | 1.53%         |
| R172K    | 36.42       | 0.43         | 1.19%         | 27.87       | 0.14      | 0.5%          |
| R172M    | 36.10       | 0.64         | 1.77%         | 27.88       | 0.17      | 0.6%          |
| R172W    | 36.32       | 0.53         | 1.46%         | 27.98       | 0.32      | 1.14%         |
| R172S    | 34.29       | 1.31         | 3.82%         | 27.97       | 0.14      | 0.51%         |
| R172Q    | 35.79       | 0.72         | 2.0%          | 27.82       | 0.12      | 0.42%         |
| R140Q    | 35.74       | 0.96         | 2.68%         | 29.64       | 0.46      | 1.56%         |
| R140W    | 34.78       | 0.83         | 2.38%         | 29.48       | 0.36      | 1.22%         |
| R140L    | 35.56       | 0.61         | 1.73%         | 29.45       | 0.33      | 1.14%         |
| R140G    | 36.77       | 0.54         | 1.47%         | 29.48       | 0.43      | 1.45%         |

### Bio-Rad CFX96

| Mutation | Mean FAM Ct | SD of FAM Ct | %CV of FAM Ct | Mean VIC Ct | SD VIC Ct | %CV of VIC Ct |
|----------|-------------|--------------|---------------|-------------|-----------|---------------|
| R132H    | 34.35       | 1.25         | 3.7%          | 28.99       | 0.23      | 0.8%          |
| R132C    | 32.41       | 1.20         | 3.7%          | 29.03       | 0.13      | 0.4%          |
| R132S    | 34.98       | 1.45         | 4.2%          | 29.14       | 0.15      | 0.5%          |
| R132G    | 33.15       | 1.53         | 4.6%          | 29.15       | 0.14      | 0.5%          |
| R132L    | 31.56       | 1.73         | 5.5%          | 28.87       | 0.41      | 1.4%          |
| R132P    | 34.33       | 2.31         | 6.7%          | 29.18       | 0.11      | 0.4%          |
| R132V    | 33.79       | 0.91         | 2.7%          | 29.15       | 0.14      | 0.5%          |
| R100Q    | 34.52       | 1.37         | 4.0%          | 29.12       | 0.12      | 0.4%          |
| R172K    | 34.50       | 0.56         | 1.62%         | 29.77       | 0.17      | 0.57%         |
| R172M    | 34.25       | 0.32         | 0.93%         | 29.94       | 0.14      | 0.48%         |
| R172W    | 36.58       | 0.47         | 1.28%         | 29.62       | 0.14      | 0.48%         |
| R172S    | 34.15       | 0.44         | 1.3%          | 29.94       | 0.33      | 1.1%          |
| R172Q    | 36.00       | 0.96         | 2.38%         | 29.69       | 0.16      | 0.59%         |
| R140Q    | 33.93       | 1.21         | 3.6%          | 29.02       | 0.28      | 1.0%          |
| R140W    | 33.74       | 1.07         | 3.2%          | 29.21       | 0.09      | 0.3%          |
| R140L    | 32.95       | 0.68         | 2.1%          | 29.23       | 0.16      | 0.6%          |
| R140G    | 34.24       | 1.33         | 3.9%          | 29.25       | 0.16      | 0.5%          |

## CONTACT

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For research use (RUO) in the U.S

For in vitro diagnostic use (IVD) in the European Union