In Vitro Diagnostic

1125021

*Revised in June 2021 (2nd edition)
Prepared in November 2020 (version 1)
Manufacture and marketing approval No. 30200EZX00081000

Use this product after reading this package insert well.

# BRAF Gene Mutation Detection Kit
# *therascreen* BRAF V600E Mutation Detection Kit RGQ "QIAGEN"

**[General precautions]**

1. In genetic diagnosis, exact information about the purpose/method and precision of genetic diagnosis, particularly the unavoidable limit of diagnosis, should be communicated to patients.
2. When performing tests, the latest package insert of the drug described in the column of purpose of use should be referred to.
3. Considering the possibility of a false-positive result, even if judged as a mutation of a type that can be detected by this product, thorough follow-up should be performed after administration of drug.
4. Confirm that tumor cells exist in the hematoxylin-eosin (HE)-stained specimen; the stained specimen should not be used for extraction of DNA.
5. Since this product is an in vitro diagnostic, it should not be used for other purposes.
6. This product should be used only by qualified healthcare professionals in a place equipped with specialized experimental facilities.
7. Diagnosis should be judged comprehensively by the doctor, including clinical symptoms and other test results.
8. For use other than the method and purpose of use described in this package insert, the reliability of the measurement result cannot be guaranteed. This product should be used according to the contents in the description.
9. Tumor specimens are not uniform, and even the same tumor may not show consistent results according to the site. Moreover, a non-tumor site may be included, and detection of mutation cannot be expected for the DNA sample.
10. This product should be used after carefully reading the handbook of *therascreen* BRAF V600E Mutation Detection Kit RGQ "QIAGEN", and the package insert and instruction manual of Rotor-Gene Q MDx 5plex HRM (RGQ).

**[Shape, configuration, etc. (contents of kit)]**

This product consists entirely of the following liquid components:

| | | |
|---|---|---|
| 1. | Control Reaction Mix (CTRL) (red)<br>Control forward primer<br>Control reverse primer<br>2'-Deoxyadenosine-5'-triphosphate (dATP)<br>2'-Deoxycytidine-5'-triphosphate (dCTP)<br>2'-Deoxyguanosine-5'-triphosphate (dGTP)<br>2'-Deoxythymidine-5'-triphosphate (dTTP) | 720 µL×2 |
| 2. | V600E Reaction Mix (V600E) (purple)<br>BRAF V600E forward primer<br>BRAF V600E reverse primer<br>2'-Deoxyadenosine-5'-triphosphate (dATP)<br>2'-Deoxycytidine-5'-triphosphate (dCTP)<br>2'-Deoxyguanosine-5'-triphosphate (dGTP)<br>2'-Deoxythymidine-5'-triphosphate (dTTP) | 720 µL |
| 3. | BRAF Positive Control (PC) (beige) | 250 µL |
| 4. | *Taq* DNA Polymerase (*Taq*) (mint) | 80 µL |
| 5. | Water for NTC (NTC) (white) | 1.9 mL |
| 6. | Water for Dilution (Dil) (white) | 1.9 mL |

**[Purpose of use]**

Detection of BRAF gene mutation (V600E) in the genomic DNA extracted from cancer tissues [used to assist judgment of application of combination therapy with encorafenib and cetuximab (genetically modified), or combination therapy with encorafenib, binimetinib and cetuximab (genetically modified) to patients with colon/rectal cancer]

**[Principle of measurement]**

This product detects the gene mutation of V600E of codon 600 of the BRAF gene in the genomic DNA extracted from biologically derived tissues using the real-time Polymerase Chain Reaction (PCR) method that applies the Scorpion-ARMS method.
The mutant BRAF gene in the extracted DNA is amplified together with the synthetic oligonucleotide for Internal Control (IC) by the PCR method that applies the Scorpion-ARMS method.

List of mutations to be measured with this product

| Mutation | Exon | Base mutation | COSMIC ID |
|---|---|---|---|
| V600E | 15 | 1799T>A | 476 |

***[Precautions for operation]**

1. Characteristics and method of sampling the sample to be measured
   For the sample to be measured with this product, use the DNA sample extracted from formalin-fixed paraffin-embedded (FFPE) tissues of patients with colon/rectal cancer.

   - Transport the tissue specimens by the standard pathological method to maintain the quality.
   - Store the FFPE block and slide at room temperature (15 to 25°C). The slide can be stored for up to 8 weeks before DNA detection.
   - In order to ensure sufficient DNA for analysis, FFPE sections of thickness at least 4 µm are required.
   - Use 2 sections of 4-5 µm for each extraction, and adjacent sections as needed.

2. Sample preparation method
   Extract the DNA sample from FFPE tissues according to the protocol of QIAamp DNA FFPE Tissue Kit [QIAGEN K.K.: Product No. 56404].
   - The RNase step will not be performed.
   - Only validated with the use of Xylene/Ethanol method for deparaffinization.
   - Proteinase K digestion (incubation at 56ºC) must be performed at 56ºC and for 1 hour.
   - De-crosslinking (incubation at 90ºC) must not exceed 1 hour.
   - Elute the purified genomic DNA by incubating in 60 µL of Buffer ATE (included in QIAamp DNA FFPE Tissue Kit) at room temperature for 2.5 minutes, and then centrifuge at the highest speed. Then, repeat the elution step with 60 µL of Buffer ATE.
   - Store the purified genomic DNA at -30 to -15°C.

3. Interfering substances
   When interfering substances which may have an impact on the measurement of this product were examined, there was no impact on judgment of the result. Moreover, there was no impact on measurement with necrosed tissues.

| Interfering substances |
|---|
| Paraffin |
| Xylene |
| Ethanol |
| ATL buffer solution |
| Proteinase K |
| AL buffer solution |
| AW1 buffer solution |
| AW2 buffer solution |
| Hemoglobin |

4. Cross-reactivity
   The cross-reactivity of this product to V600 mutations (V600K, V600R, V600G, V600M, V600D and V600Ec) was checked. When V600K, V600R, V600G and V600M were tested at high-level MAF using genomic DNA, no cross-reactivity was observed.
   Cross-reactivity was confirmed for V600D and V600Ec, but V600D- and V600Ec-mutations are rare mutations in the population of colon/rectal cancer (from COSMIC database).

5. Other precautions
   - If there is any substance that might interfere with PCR in the sample, a correct judgment result will not be obtained, and

therefore, attention should be paid to this.
- Close attention should be paid so that the PCR amplified product is not contaminated.
  When the Reaction Mix is set up, and when PC and the DNA sample are added, it is recommended to use the respective dedicated pipets.
  Moreover, preparation and dispensing of the Reaction Mix should be performed at a place different from addition of the DNA template.
- Correct examination should be performed while paying attention to error in sampling and operation.
- If lower than the minimum detection sensitivity even if the target DNA is present in the sample, the result may be judged to be negative. Therefore, care should be taken.

**[Dosage and administration (operating method)]**

1. Method of preparation of reagent
   1) Thaw all reagents completely at ordinary temperature
   (15 to 25℃) for at least 1 hour before use, then mix gently by inversion 10 times, and spin down with a centrifuge.
   2) Preparation of Master Mix
   Mix Control Reaction Mix or V600E Reaction Mix and *Taq* DNA Polymerase to prepare the Master Mix, and mix by pipetting 10 times slowly. The amount required for preparation of the Master Mix is as shown in the table.

| Component reagents | Amount |
|---|---|
| Reaction Mix | 19.5 µL × (n+X)* |
| *Taq* DNA Polymerase [Taq] | 0.5 µL × (n+X)* |
| Whole amount | 20.0 µL/1 reaction |

*N= Number of reactions (sample + control). In preparing the Master Mix, prepare n+X (excess sample) so as to perform sufficient treatment for setting of PCR (however, X=1 when control is added to 4 or less samples, and X=2 when control is added to 5 or more samples).

- Initially place each Reaction Mix (component reagents 1-2) in a tube, and then add *Taq* DNA Polymerase.
- Return *Taq* DNA Polymerase to room temperature (15 to 25℃) before each use, and spin down.
- Dispense *Taq* DNA Polymerase at a level where the tip comes just below the liquid surface so that excess enzyme does not attach to the tip.
- Do not vortex *Taq* DNA Polymerase or the solution (Master Mix) containing *Taq* DNA Polymerase. There is a risk that enzymes may be inactivated.
- After preparing the reagents, prepare the PCR reactions and start measurement immediately.
- Use the prepared reagents immediately. If the reagents are not used immediately, use them within 4 hours at room temperature including the time for PCR set-up, and within 12 hours at 2 to 8℃.
- For each Reaction Mix, it is necessary to protect from light to maintain appropriate activity and to avoid photomodification.
- Up to 24 samples can be evaluated at the volume of each Reaction Mix.
- In order to use this product efficiently, perform measurement with at least 6 samples.

   3) BRAF Positive Control
   Use as it is.

   4) Water for NTC
   Use as it is.

   5) Water for Dilution
   Use as it is.

2. Other necessary apparatuses, equipment, samples, etc.
   1) Reagent
   - Genomic DNA preparation kit: QIAamp DNA FFPE Tissue Kit (QIAGEN K.K., product No. 56404)

   2) Consumables
   - 1.5-mL or 2-mL microtube (for dissolution)
   - 1.5-mL microtube with a cap (for elution)
   (Eppendorf [Eppendorf Safe-Lock Tubes, 1.5 mL, catalog No. 0030 120.086] or Sarstedt [Safety Cap, catalog No. 72.690] is recommended)
   - 0.1-mL Strip tube and Cap 72 for well rotor (product for use only with RGQ)
   - Sterile pipet tip (with a hydrophobic filter)
   - Nuclease-free low-DNA bound microcentrifuge tube (recommended for preparation of Master Mix)
   - Micropipet

   3) Equipment
   - Gene analyzer Rotor-Gene Q MDx 5plex HRM
   (Medical device notification No.: 13B2X10223000004)

   and consumables*

   - Thermomixer or water bath
   (incubation can be performed at 56℃ and 90℃)
   - Centrifuge
   - Vortex mixer

   *Use the consumables for RGQ.

3. Operating methods

Prepare the sample with reference to "**[Precautions for operation]** 2. Sample preparation method". In so doing, pay close attention to cross-contamination. For a DNA sample extracted according to the sample preparation method, start measurement immediately. If measurement is not performed immediately after extraction, the sample can be stored at -15℃ to -30℃ for 8 weeks before the start of measurement.

Before the start of operation, read [General precautions] and [Precautions for use or handling] thoroughly. Start measurement after familiarization with handling referring to the handbook of this product, the package insert and instruction manual for RGQ, and then start measurement. The test consists of 2 steps: sample evaluation test and mutation detection test.

1) Sample evaluation test

① According to "1. Method of preparation of reagent", mix Control Reaction Mix and *Taq* DNA Polymerase, and prepare the required amount of Master Mix.
Pipet up and down 10 times, and mix the Master Mix thoroughly. Place an appropriate quantity of Strip tube (tube) in a loading block according to the layout in the table. Add 20 µL of Master Mix to each tube immediately.

Arrangement of samples on loading block

| Reaction Mix | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Control | 1( PC) | 9 | 17 | – | – | – | – | – | – |
| Control | 2 (NTC) | 10 | 18 | – | – | – | – | – | – |
| Control | 3 | 11 | 19 | – | – | – | – | – | – |
| Control | 4 | 12 | 20 | – | – | – | – | – | – |
| Control | 5 | 13 | 21 | – | – | – | – | – | – |
| Control | 6 | 14 | 22 | – | – | – | – | – | – |
| Control | 7 | 15 | 23 | – | – | – | – | – | – |
| Control | 8 | 16 | 24 | – | – | – | – | – | – |

② Perform the following procedures immediately.
   a) Add 5 µL of NTC to position 2, and close the cap.
   b) Add 5 µL of the sample to each of positions 3-24, and close the caps.
   c) Add 5 µL of PC to position 1, and close the cap.
   Note) Put a mark on the cap of the tube to indicate the direction of loading to RGQ.

③ After capping all tubes, check the filling level of the tube visually, and confirm that the solution is filled in all tubes.

④ Overturn all tubes 4 times for mixing, and set the tubes to the rotor disc.

⑤ If there is any empty site on the rotor disc, load an empty tube with cap at the position.

⑥ Mount the rotor disc to RGQ, and start measurement according to the instruction of the Rotor-Gene Assay Manager. Refer to the handbook of this product and the package insert and instruction manual of RGQ for detailed operations.

2) Mutation detection test

① According to "1. Method of preparation of reagent", mix V600E Reaction Mix, Control Reaction Mix and *Taq* DNA Polymerase, and prepare the required amount of Master Mix.

② Pipet up and down 10 times, and mix the Master Mix thoroughly. Place an appropriate quantity of Strip tube (tube) in a loading block according to the layout in the table. Add 20 µL

of Master Mix to each tube immediately.

Arrangement of Reaction Mix on loading block (number indicating the position of the loading block and the final position on the rotor disc. The **bold type** indicates the sample number),

| Reaction Mix | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **PC** | **3** | **7** | **11** | **15** | **19** | **23** | – | – |
| Control | 1 | 9 | 17 | 25 | 33 | 41 | 49 | – | – |
| *BRAF* | 2 | 10 | 18 | 26 | 34 | 42 | 50 | – | – |
| | **NTC** | **4** | **8** | **12** | **16** | **20** | **24** | – | – |
| Control | 3 | 11 | 19 | 27 | 35 | 43 | 51 | – | – |
| *BRAF* | 4 | 12 | 20 | 28 | 36 | 44 | 52 | – | – |
| | **1** | **5** | **9** | **13** | **17** | **21** | – | – | – |
| Control | 5 | 13 | 21 | 29 | 37 | 45 | – | – | – |
| *BRAF* | 6 | 14 | 22 | 30 | 38 | 46 | – | – | – |
| | **2** | **6** | **10** | **14** | **18** | **22** | – | – | – |
| Control | 7 | 15 | 23 | 31 | 39 | 47 | – | – | – |
| *BRAF* | 8 | 16 | 24 | 32 | 40 | 48 | – | – | – |

③ Perform the following procedures immediately.
a) Add 5 µL of NTC to positions 3-4, and close the caps.
b) Add 5 µL of each sample to positions 5-6, 7-8 and 9-10 in the required amount, and close the caps.
c) Add 5 µL of PC to positions 1-2, and close the caps.
Note: It is necessary to measure each sample with both Control Reaction Mix and V600E Reaction Mix. Also, put a mark on the cap of the tube to indicate the direction of loading to RGQ.

④ After capping all tubes, check the filling level of the tube visually, and confirm that the solution is filled in all tubes.

⑤ Overturn all tubes 4 times for mixing, and set the tubes to the rotor disc.

⑥ If there is any empty site on the rotor disc, load an empty tube with cap at the position.

⑦ Mount the rotor disc on RGQ, and start the test according to the instruction of the Rotor-Gene Assay Manager. Refer to the handbook of this product and the package insert and instruction manual of RGQ for detailed operations.

3) Re-inspection
If the test failed, it can be performed one more time.
① Sample evaluation test
a) When the DNA input amount is low
If the sample evaluation test failed because the DNA input amount is too low, the sample will be judged to be invalid, and the flag shown in the table below will be displayed.

Evaluation flag of sample with low DNA input amount

| Flag | Description |
|---|---|
| NO_VALUE | The value assumed to be obtained has not been obtained. |
| ABOVE_ACCEPTED_RANGE | The target value is higher than the specified range. There is a possibility that the Ct value exceeds CWR*, or exceeds the specified value of IC or PC. |

*CWR・・・Control working range. The range of CWR is the Ct value from 20.95 to 33.00.

Re-inspect the sample from the sample evaluation test.
If the sample evaluation test failed because the second DNA input amount is also too low, it is necessary to perform re-extraction from 2 sections derived from the same sample. Then, perform the sample evaluation test up to the second time as shown above.
If the sample does not show valid results even if all the above re-inspections are performed, consider the sample to be inappropriate for test, and report it to be invalid.

Note) For invalid samples, a sample can be prepared from the same patient and examined.

b) When the DNA input amount is high
If the sample evaluation test of a certain sample failed because the DNA input amount is too high, the sample will be judged to be invalid, and the flag shown in the table below will be displayed.

Evaluation flag of sample with high DNA input amount

| Flag | Description |
|---|---|
| SAMPLE_CTRL_HIGH_CONC | Since the DNA concentration is too high, the sample is invalid. In order to increase the control Ct value, the sample should be diluted. For dilution, calculation should be performed assuming that, if diluting with Water for Dilution included in the kit to 1:2, Ct increases by 1.0. If the sample was diluted, a new set-up should be performed to repeat the sample evaluation test. |

Dilute the sample with Water for Dilution so as to be included in CWR. By diluting the sample at 1:2, consider that the Ct value increases by about 1.0. If the sample evaluation test was performed on the sample after dilution and the flag is displayed after dilution, repeat dilution as appropriate.
If appropriate diluted samples were obtained by the procedures for the above sample evaluation test, perform the mutation evaluation test with the sample after dilution.

② Mutation detection test
a) When the DNA input amount is low
If an invalid result is obtained because the DNA input amount was too low even if the sample passed the sample evaluation test and moves to the mutation detection test, either of two flags similar to the sample evaluation test will be displayed.
It may be necessary to inspect the sample again. If the same flag is displayed again in the re-inspection of the mutation detection test, it is necessary to perform re-extraction of the DNA sample from two sections derived from the same sample. The newly extracted DNA sample should be tested starting from the sample evaluation test again.
If the mutation detection test was invalid twice even with the re-extracted DNA sample, additional inspection will not be performed.

Note) For invalid samples, a sample can be prepared from the same patient and examined.

③ Invalidity due to IC
If the test became invalid due to IC after the mutation detection test (displaying the flag SAMPLE_INT_CTRL_FAIL), it is necessary to dilute the sample with Water for Dilution with reference to the table below. It is necessary to perform the mutation detection test of the diluted sample from the start.

| Control Ct value | Recommended dilution factor |
|---|---|
| <30.00 | 8 |
| ≥30.00 | 4 |

If the DNA input amount was too low after dilution at 1:8, it is necessary to perform the mutation detection test again using a thinner dilution factor (1:4).
If the SAMPLE_INT_CTRL_FAIL flag is displayed even after the second mutation detection test after dilution of sample, it is necessary to perform re-extraction of the DNA sample from two sections derived from the same sample. The newly extracted DNA sample should be tested starting from the sample evaluation test again.
After retest of the sample evaluation test and retest of the mutation detection test, if the re-extracted DNA sample was invalid due to IC even after the second evaluation was performed, the result will be reported to be invalid, and additional inspection will not be performed.

Note) For invalid samples, a sample can be prepared from the same patient and examined.

**[Method of judgment of measurement result]**
Perform the reaction to amplify and detect each mutant specifically and the control reaction to detect the wild type (exon 3) simultaneously on each sample, monitor each amplified product in real time for each cycle, and prepare the respective amplification curves.
From the prepared amplification curve, determine the number of cycles at which the fluorescence intensity increases above a certain

level, and use it as the Ct (Cycle threshold) value. Calculate the difference (⊿Ct) between the Ct value (mutation detection Ct value) determined in each reaction, and the Ct value (control Ct value) determined in the control reaction. If measured using RGQ, the Ct value will be calculated automatically.

⊿Ct value = Mutation detection Ct value - Control Ct value

The cutoff value in the mutation detection test of this product is as described in the table below.

| Mutation | Cutoff value (⊿Ct value) |
|---|---|
| V600E | ≦ 7.0 |

The measurement result is any of "BRAF Mutation detected" (positive), "No Mutation Detected" (negative), or "Invalid".
If the ⊿Ct value exceeds the cutoff value, judge the result to be negative, if it is below the cutoff value, judge the result to be positive, and if the criteria for detection are not satisfied in other cases, judge the result to be invalid.

Determination of threshold value and judgment of result are performed based on the following control (PC, NTC, IC) acceptance criteria. (If deviated from the acceptance criteria, the measurement will be judged to be invalid).

1) Analysis of test
If the control measurement result is within the acceptable range, the measurement will be judged to be valid.

Acceptance standard of control

| Reaction Mix Type | Well | Channel | Acceptance standard of Ct value (Ct) |
|---|---|---|---|
| Control Reaction Mix (Control) | PC | Green (FAM)* | 27.82-33.85 |
| V600E Reaction Mix | PC | Green (FAM)* | 27.49-33.51 |
| Both Reaction Mix | NTC | Green (FAM)* | NA |
| Both Reaction Mix | NTC | Yellow (HEX)** | 32.53-38.16 |

Acceptable standard of IC

| Assay | Channel | Acceptable standard (Ct) |
|---|---|---|
| Internal Control | HEX* | 32.53-38.16 |

*FAM：Carboxyfluorescein, green fluorescent pigment
**HEX：Hexachlorofluorescein, yellow fluorescent pigment

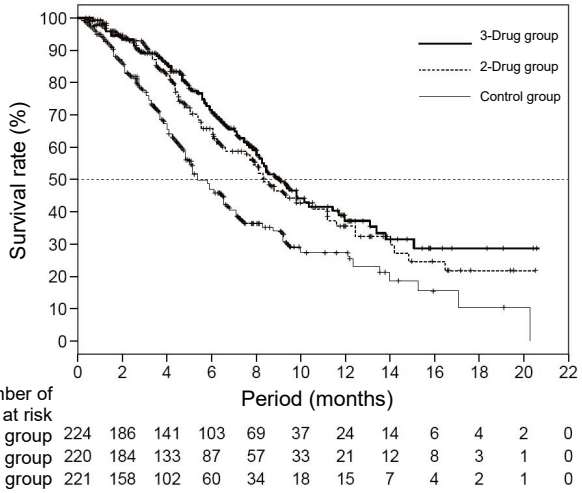
2) Precautions for interpretation of result
（1）Since there is the possibility that a sample containing a substance inhibiting the PCR reaction may give a false-negative, care should be taken.
（2）Diagnosis should be judged comprehensively by the doctor, including clinical symptoms and other test results.
（3）Measurement with this product should be performed by a person in charge who has received training in laboratory tests and the equipment to be used.

**[Clinical significance]**

International joint phase III study (ARRAY-818-302 Study)

In 665 patients with advanced/relapsed colon/rectal cancer where it is impossible to perform curative resection, who have the BRAF V600E mutation exacerbated after primary treatment or secondary treatment (including 20 Japanese patients), the efficacy and safety of (1) concomitant administration of encorafenib,[*4] binimetinib[*5] and cetuximab[*2] (3-drug group) or (2) concomitant administration of encorafenib[*2] and cetuximab[*2] (2-drug group), were examined in comparison with concomitant administration of FOLFIRI[*1] or cetuximab[*2] or concomitant administration of irinotecan[*3] and cetuximab[*2] (control group). The results[*6] of primary endpoints, overall survival and response rate, are shown below. In comparison with the control group, the 3-drug group and 2-drug group statistically significantly extended the overall survival, and the response rate was statistically significantly high (data cutoff on February 11, 2019).

*1: Considering 2 weeks as a cycle, on Day 1, (1) irinotecan 180mg/m$^2$ was intravenously administered over 90 minutes, (2) folinate 400mg/m$^2$ was intravenously administered over 120 minutes, (3) fluorouracil 400mg/m$^2$ was administered by bolus intravenous injection, and then fluorouracil 2,400mg/m$^2$ was intravenously administered over 46 to 48 hours.
*2: 400mg/m$^2$ was intravenously administered over 120 minutes only the first time, and then 250mg/m$^2$ was intravenously administered over 60 minutes every week.
*3: Considering 2 weeks as a cycle, 180mg/m$^2$ was intravenously administered over 90 minutes.
*4: 300 mg was administered once a day.
*5: 45 mg was administered twice a day.
*6: In this study, as the main purpose, the superiority of response rate and overall survival in the 3-drug group to those in the control group was verified, and then, as a secondary purpose, according to the hierarchical test procedure, an analysis of overall survival and response rate in the 2-drug group were performed in comparison with those in the control group.




| | | 3-Drug group | 2-Drug group | Control group |
|---|---|---|---|---|
| Overall survival | Number of patients | 224 | 220 | 221 |
| | Median value (months) [95% confidence interval] | 9.0 [8.0, 11.4] | 8.4 [7.5,11.0] | 5.4 [4.8, 6.6] |
| | Hazard ratio[*7] [95% confidence interval] p-value[*8] | 0.52 [0.39, 0.70] <0.0001 | 0.60 [0.45, 0.79] 0.0002 | - |
| Response rate[*9] | Number of patients[*10] | 111 | 113 | 107 |
| | Response rate (%) [95% confidence interval] | 26.1 [18.2, 35.3] | 20.4 [13.4. 29.0] | 1.9 [0.2, 6.6] |
| | p-value[*11] | <0.0001 | <0.0001 | |

*7: Stratified Cox proportional hazard model (comparison with control group)
*8: Stratified log-rank test (comparison with control group)
*9: CR or PR by central judgment based on the RECIST guideline version 1.1
*10: 331 patients first registered in this study were analyzed
*11: Cochran-Mantel-Haenszel test (comparison with control group)

**[Performance]**

1. Performance test
1) Sensitivity test
When the positive control was measured with Control Reaction Mix and V600E Reaction Mix, the Ct values were 27.82-33.85 and 27.49-33.51, respectively.

2) Accuracy test
When the positive control was measured with Control Reaction Mix and V600E Reaction Mix, the Ct values were 27.82-33.85 and 27.49-33.51, respectively.
When the negative control was measured with Control Reaction Mix and V600E Reaction Mix, the result was No Amplification for both.

3) Repeatability test
When the positive control was measured twice with Control Reaction Mix and V600E Reaction Mix respectively, the Ct

values were 27.82-33.85 and 27.49-33.51, respectively.
When the negative control was measured twice with Control Reaction Mix and V600E Reaction Mix respectively, the result was No Amplification for both.

・Control substances
The negative control is “Water for NTC” (purified water).
The positive control is “BRAF Positive Control” which is a long oligonucleotide.

2. Minimum detection sensitivity
Minimum detection sensitivity test
Using FFPE clinical samples prepared to high, middle and low DNA concentrations, measurement with this product was performed. The minimum detection sensitivity (LoD value) was defined as the minimum value for the sample which can be detected at the 95% confidence interval determined by probit analysis. The results are as shown in the table below.

| DNA concentration | Range of Ct value | LoD value (MAF concentration) |
|---|---|---|
| High | 20.95≦Ct ≦25.00 | 2% |
| Middle | 25.00< Ct ≦29.38 | 3.5% |
| Low | 29.38< Ct ≦33.00 | 7.8% |

3. Clinical study
(1) A study of concordance rate between CTA and this product using samples in the BEACON CRC Study (bridging study)
Utilizing samples obtained from patients in the BEACON CRC Study, a study to be evaluated, the clinical efficacy of this product as well as the concordance rate were verified by overall comparison of the product with CTA* (Clinical Trial Assay) and LDT** (Laboratory Developed Test).

*CTA: A test used in the BEACON Study and performed by the central inspection agency. This product is one in which the CTA parameter was improved.
**LDT: A test in the BEACON Study and performed in the study institutions.

(2) Comparison with CTA (bridging study)
The overall percent agreement between this product and CTA using the clinical samples of 1285 patients was 99.84%
(95% CI: 99.44%, 99.98%).

| Concordance rate of the number of detected mutations between this product and CTA | | | |
|---|---|---|---|
| CTA | This product | | |
| Presence or absence of detection | Detected | Not detected | Total |
| Detected | 770 | 1 | 771 |
| Not detected | 1 | 513 | 514 |
| Total | 771 | 514 | 1285 |

Overall percent agreement: 99.84% (1283/1285)
Positive percent agreement: 99.87% (770/771)
Negative percent agreement: 99.81% (513/514)

(3) Comparison with LDT (bridging study)
The overall percent agreement between this product and LDT using the clinical samples of 661 patients was 96.82%
(95% CI: 95.18%, 98.02%).

| Concordance rate of the number of detected mutations between this product and LDT | | | |
|---|---|---|---|
| LDT | This product | | |
| Presence or absence of detection | Detected | Not detected | Total |
| Detected | 520 | 15 | 535 |
| Not detected | 6 | 120 | 126 |
| Total | 526 | 135 | 661 |

Overall percent agreement: 96.82% (640/661)
Positive percent agreement: 98.86% (520/526)
Negative percent agreement: 88.89% (120/135)

***[Precautions for use or handling]**

1. Precautions for handling (prevention of hazard)

1) The sample should be handled with the same precautions as those when there is a risk of infection by HBV, HIV, HCV, etc.
2) In the examination, the requisite protective equipment such as disposable gloves, white garments, and protective glasses should be worn to avoid the risk of infection.
3) For more information, please consult the appropriate safety data sheets (SDSs). These are available online in convenient and compact PDF format at www.qiagen.com/safety where you can find, view and print the SDS for each QIAGEN kit and kit component.
4) Do not suck a pipet with the mouth.
5) If reagent erroneously adheres to the skin and mucosa, it should be washed out with plenty of water immediately.
6) If reagent was spilled, it should be wiped off after diluting with water.
7) If the extracted sample was spilled on the floor, etc., it should be wiped off thoroughly using disinfectants such as hypochlorite agents (effective chlorine concentration 5000 ppm, 0.5%). When wiping off, actions to protect the hands, such as wearing rubber gloves, should be taken.
8) Drinking and eating or smoking at a place where samples and this product are handled should be avoided.
9) The apparatus used to handle samples should be sterilized by heating at 121℃ for at least 20 minutes using an autoclave, or disinfected by immersing in a hypochlorite agent (effective chlorine concentration 5000 ppm, 0.5%) for at least 1 hour. The air should be ventilated thoroughly during these operations.

2. Precautions for use
1) The therascreen BRAF V600E RGQ PCR Kit is shipped on dry ice and must still be frozen on arrival. If the therascreen BRAF V600E RGQ PCR Kit is not frozen on arrival, the outer packaging has been opened during transit, or the shipment does not contain a packing note or reagents, please contact the QIAGEN Technical services or local distributors (visitwww.qiagen.com).
2) Dedicated reagents and consumables should be used, and their containers and accessories should not be used for other purposes. All the reagents included with this product are dedicated for use with this product. In order to maintain performance, other reagents should not be used as substitutes.
3) Each reagent is diluted to the optimum concentration. Further dilution should not be performed because the reaction may become poor.
4) In order to avoid the risk of false-negativity, the volume of reaction liquid should not be less than 25 μL.
5) Reagents must be stored according to their storage method, and those stored by a method other than the designated one or which have passed their expiration date (expiration date indicated on the outer box) must not be used.
6) Since there is a risk that it may affect performance, reagents of different lots or residual reagents should not be mixed.
7) Only *Taq* DNA Polymerase in this kit should be used, and the *Taq* DNA Polymerase of different kits or different companies should not be used.
8) Since reagents are verified for manual use, input of dead volume is required in the case of automatic measurement, and the number of reactions may decrease.
9) All reagents should be kept at ordinary temperature (15℃ to 25℃) for at least 1 hour, and used after returning to ordinary temperature. After use, reagents should be stored at -30℃ to -15℃ again.
10) For this product, freezing and thawing should not be repeated more than 6 times.
11) Strong light should not be irradiated to any reagents during storage or reaction. It is necessary to protect all Scorpions from light to maintain performance and to avoid photomodification.
12) For all reagents, contamination with microorganisms should be avoided in opening or dispensing.
13) The PC and samples should be stored and extracted away from other reagents, and added to Reaction Mix at a place distant from other reagents.
14) Preparation and dispensing of Reaction Mix should be performed at a place distant from the DNA template.
15) Preparation of PCR reactions should be performed on a clean bench equipped with ultraviolet irradiation equipment. Pipets should be stored on this clean bench at all times. DNA after amplification should not be taken into an area to prepare a PCR reaction. For dispensing of samples, moreover, disposable tips equipped with a hydrophobic filter should be used.

16) In order to prevent contamination, the lid of the tube should be closed immediately after addition of sample. The lid of the reaction tube after PCR reaction should not be opened.
17) Division of the inspection area, dedicated use of pipets, and cleaning of apparatus and laboratory tables with hypochlorous acid (effective chlorine concentration 5000 ppm, 0.5%) should be implemented thoroughly.
18) In handling this product, contamination by microorganisms and nucleolytic enzymes should be avoided. Even if a small amount of DNase contained in sweat or saliva contaminates the sample, the DNA may be degraded, and errors may occur in the measurement result.
19) For details of operation, refer to the handbook and the package insert, and the instruction manual of RGQ.
20) Prior to use, make sure that instruments have been checked and calibrated according to the manufacturer's recommendations.
21) The loading block should be decontaminated before use, and dried before use.
22) The product number should be checked at the time of use.

3. Precautions for disposal
1) Waste liquids generated by measurement should be sterilized or disinfected in a similar manner to samples. They should be discarded according to the regulations for waste products.
2) When containers are discarded after use, they should be handled separately from medical waste products and industrial waste products according to the regulations for waste products.
3) The nucleic acid sample and amplified DNA after genetic testing should be mixed with hypochlorite agents to the effective chlorine concentration of 5000 ppm, 0.5%, allowed to stand overnight to destroy DNA, and then discarded.
4) Pipet tips and plastic containers after handling DNA should be immersed in hypochlorite agents (effective chlorine concentration 5000 ppm, 0.5%) overnight to destroy DNA, and then incinerated or discarded as medical waste products.

**[Storage methods and expiration period]**
1. Storage method: Protect from light, store at -30℃ to -15℃
2. Expiration period: 12 months (the expiration date is indicated on the outer box)

**[Packaging unit]**

| Product number | Content of package | Packaging unit |
|---|---|---|
| 874854 | *therascreen* BRAF V600E Mutation Detection Kit RGQ "QIAGEN" | 24 tests |

For details of each component reagent, refer to **[Shape, configuration, etc. (contents of kit)]**.

**[References]**

1) C.R.Newton, et al. Analysis of any point mutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Research, Volume 17, Number 7 1989: 2503-2516
2) David Whitcombe, et al. Detection of PCR products using self-probing amplicons and fluorescence. Nature Biotechnology, Volume 17, August 1999: 804-807.
3) Ono Pharmaceutical Co., Ltd.: Results of International Joint Phase III (ARRAY-818-302) Study
(Internal data; approved on November 27, 2020, CTD2.7.6.6)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