REF IDSARSCOV-N/E-100
IDSARSCOV-N/E-500

Instructions for use

ID™ SARS-CoV-2 / N501Y/ E484K Quadruplex

Σ 100 tests
500 tests

Qualitative detection system of **RNA SARS-CoV-2 detection** and **screening for N501Y and E484K mutations** by real-time RT-PCR assay

---

CE

IVD

ID SOLUTIONS, 310, rue Louis Pasteur – Grabels - FRANCE
Tel : +33 (0)4 67 79 72 60 – Fax : + 33 (0)4 67 45 36 95
www.id-solutions.fr - E-mail : info@id-solutions.fr

SOMMAIRE

1 KIT PRESENTATION

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit is an in vitro diagnostic test based on real-time RT-PCR (or RT-qPCR) technology for the qualitative detection of viral RNA of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and for screening of N501Y and E484K mutations of the SPIKE gene.

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex kit** is intended for clinical laboratories receiving nasopharyngeal and oropharyngeal samples by swab, sputum samples (samples taken by an authorized healthcare professional). Thus, a nucleic acid extraction must be carried out prior to proceeding with the test. The kit should be used with a real-time PCR thermocycler. The performance of this reagent is guaranteed only if the laboratories comply with the ISO15189 standard.

The results of the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit allow to detect the presence of the RNA of SARS-CoV-2, to identify whether RNA carries the **N501Y and / or E484K mutations** and therefore to help in the diagnosis of COVID-19.

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit should only be used by laboratory professionals who have received instructions and training in real-time PCR techniques related to the thermocycler used and in vitro diagnostic procedures.

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit is intended for *in vitro* diagnostic use.

2 TEST PRINCIPLE

1. SAMPLE TYPES

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit should be used with nucleic acid samples previously extracted from nasopharyngeal, oropharyngeal swabs or sputum samples.

2. EXTRACTION OF NUCLEIC ACIDS

Nucleic acids of the original genotype and SARS-CoV-2 variants should be extracted from samples before amplification by RT-qPCR.

It is important to ensure that the nucleic acid extraction system used is compatible with real-time PCR technology. The quality of the extracted RNAs is essential to ensure the performance of the entire test.

The suitability of the nucleic acid extraction procedure for use with the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit must be validated by the user.

3. REAL-TIME AMPLIFICATION AND DETECTION

- **Principle**

The technology used for the test is real-time RT-PCR, which firstly allows the reverse transcription of the extracted RNA into complementary DNA (cDNA), then secondly, the amplification of specific genome sequences. The presence of nucleic acids is then detected by an increase in fluorescence due to the hydrolysis of specific probes during the amplification step (5 'nuclease hydrolysis probe technique).

- **Amplified sequences of SARS-CoV-2 and mutations**

The primers used in the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** test are used to amplify:

- Two specific target sequences of the SARS-CoV-2 coronavirus at the level of the sequence encoding the nucleocapsid protein (called N) and of the sequence encoding the RNA-dependent RNA polymerase (called RdRP).
- Two sequences encoding the spike protein (SPIKE - S) at the region encoding the RBD (binding domain receptor) to detect the presence of N501Y and E484K mutations.

ID™ SARS-CoV-2 / N501Y / E484K Quadruplex is a qualitative quadruplex system that allows, for each sample, the simultaneous amplification of RNA targets in only one reaction. Primers and double-labeled probes (hydrolysis probe) are designed in a specific region to allow sensitive and specific amplification and detection of SARS-CoV-2 RNA and the presence of N501Y and/or E484K mutations if present in the sample. The test also detects a human genetic sequence to control the presence of the sample.

The presence of nucleic acids is detected by an increase in fluorescence due to the hydrolysis of the probes during amplification.

The fluorescence signals for the amplification of the specific probes of the N and RdRP are measured in the FAM™ channel (N and RdRP target); the signal for the detection of the N501Y mutation is measured in the VIC®/HEX™ channel (SPIKE N501Y target), the signal for the detection of the E484k mutation is measured in the ROX / CAL Fluor® Red 610 channel (SPIKE E484K target), and the fluorescence signal for the endogenous target is measured in the Cy5 channel (Endo target).

- **Real-time PCR instruments**

The **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit has been developed and validated for use with the real-time PCR thermocyclers listed in Table 1.

Table 1. LIST REAL-TIME PCR INSTRUMENTS VALIDATED WITH E KIT **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex**

Maker	Model
Applied Biosystems	QuantStudio 5 ™
rock	LightCycler 480 (System II) *
Bio-Rad	CFX96 Touch real-Time PCR detection System
Agilent	AriaMx Real-time PCR

* A color compensation of the four channels FAM™, VIC ®/HEX ™, ROX/CAL Fluor® Red 610 and Cyanine 5 (Cy5) is required when using the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit.

⚠ Ensure that instruments and equipment have been checked and calibrated according to the manufacturer's recommendations.

4. CONTROLS

- **Amplification controls**

The following controls should be used for each run of analysis:

Positive Amplification Control (PAC)

This control is present in the kit (PAC-IDSARSCOV2-N/E). It contains a mixture of synthetic DNA from each target, calibrated to the values mentioned in the quality control sheet of the corresponding lot.

It validates the amplification step of each target.

Negative Amplification Control (NAC)

This control is not present in the kit. It corresponds to the deposit of 8 µL of amplification reaction mixture (ARM-IDSARSCOV2-N/E) and 5 µL of nuclease-free water.

The control should be amplified at the same time and according to the same protocol as the samples extracted from patients. It makes it possible to verify the absence of contamination during the amplification step.

3 REAGENTS AND MATERIAL

1. KIT COMPOSITION

The **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** Kit is available under two references: IDSARSCOV-N/E--100 (100 reactions) and IDSARSCOV-N/E-500 (500 reactions). The kit composition is presented in the table below:

TABLE 2. COMPONENTS OF **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** KIT

COMPONENT	IDSARSCOV-N/E-100	IDSARSCOV-N/E-500	COLOR	DESCRIPTION
PAC-IDSARSCOV2-N/E Positive Amplification Control	100 µL 1 tube	100 µL 1 tube		Mixture of specific nucleic acids of SARS-CoV-2, N501Y mutation, E484K mutation and human endogenous control.
ARM-IDSARSCOV2-N/E Mélange Réactionnel d'Amplification	800 µL 1 tube	800 µL 5 tubes		Reaction mixture containing Reverse Transcriptase, Taq polymerase, primers, and hydrolysis probes for the detection of SARS-CoV-2, N501Y mutation, E484K mutation and human endogenous control.

2. PREPARATION AND STORAGE CONDITIONS OF THE REAGENTS

The **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit must arrive frozen upon receipt. If any component of the kit is not frozen on arrival or if one of the tubes has been damaged during transport, please contact **ID SOLUTIONS.**

Before and after opening the kit	Reagents should be stored between -16 °C and -26 °C and protected from light
After thawing	The reagents must be kept on a cooling block (+2 °C/+8 °C) or on ice during all their use.
Before use	The reagents must be completely thawed, then they must be homogenized.
After use	The reagents must be replaced as quickly as possible between -16°C and -26°C protected from light.

TABLE 3. CONDITIONS OF PREPARATION, STORAGE AND USE OF REAGENTS OF THE KIT **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex**

DÉSIGNATION	NAME	PRÉPARATION	CONDITIONS OF STORAGE AND USE
PAC-IDSARSCOV2-N/E	Positive Amplification Control	Ready-to-use	Do not exceed 3 freeze/thaw cycles
ARM-IDSARSCOV2-N/E	Amplification Reaction Mix	Ready-to-use	Do not exceed 3 freeze / thaw cycles Must be stored away from light (storage, during thawing). Reagent is sensitive to temperature variations. Once thawed, the reagent should be used immediately and then returned to the freezer at -16 ° C to -26 ° C after use. Do not store the tube at room temperature or +4 ° C. Use an ice pack (+ 2 ° C / + 8 ° C) or ice throughout its handling before returning it to the freezer as soon as possible.

When stored under the specified storage conditions, the kit is stable until the stated expiration date.

It is recommended to store the PCR reagents in a pre-amplification area (ARM-IDSARSCOV2-N/E) and the control (PAC-IDSARSCOV2-N/E) in a post-amplification area (manipulation of DNA).

The kit is stable for 3 months after opening in the absence of contamination.

⚠ **The ARM- IDSARSCOV2-N/E is very sensitive to temperature variations, strictly follow the instructions for storage and use conditions in the table above.**

3. MATERIAL NEEDED BUT NOT PROVIDED IN THE KIT

- Systems or kits suitable for nucleic acid extraction, follow the manufacturer's instructions (see principle of the test),
- Real-time PCR thermocyclers validated with the kit (see test principle),
- Centrifuge for tubes or plate,
- 96- or 384-well plates or reaction tubes with adhesive films or stoppers for closure adapted to each validated thermocycler,
- Refrigerated rack or ice,
- Precision pipettes and nuclease-free tips with filters adapted to the volume to be pipetted,
- Distilled or nuclease-free water (DNase and RNase).

4 INSTRUCTIONS AND PRECAUTIONS OF USE

The use of this product is limited to qualified personnel trained in real-time PCR techniques and *in vitro* diagnostic procedures. Good laboratory practice should be followed.

Please read the instructions before starting the test.

1. HYGIENE AND SAFETY GUIDELINES

- **Chemical hazard**

The following components (listed in the table below) of **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** contain hazardous substances.

- Wear gloves and safety glasses and follow the safety advice in this section.

Note: Dangerous substances do not have to be accompanied by hazard statements and precautionary statements when quantities are less than 125 mL (EU 1272/2008 Annex I 1.5.2).

Component	Contains	Hazard pictograms & signal words	Hazard statements	Precautionary statements
ARM-IDSARSCOV2-N/E	Tetramethylammonium chloride CAS 75-57-0	 WARNING	H302 H371 H412	P308+P311 P260 P264 P270 P301+P312 P330 P405 P501

Hazard statements : H302 - Harmful if swallowed.
H371 - May cause damage to organs (central nervous system) (if swallowed).
H412 - Harmful to aquatic life with long lasting effects.

Precautionary statements : P308+P311 - IF exposed or concerned: Call a POISON CENTER or doctor.
P260 - Do not breathe dust/fume/gas/mist/vapours/spray.
P264 - Wash hands, forearms and face thoroughly after handling.
P270 - Do not eat, drink or smoke when using this product.
P301+P312 - IF SWALLOWED: Call a POISON CENTRE or doctor if you feel unwell.
P330 - Rinse mouth.
P405 - Store locked up.
P501 - Dispose of contents/container to hazardous or special waste collection point, in accordance with local, regional, national and/or international regulation.

-For more information, see the appropriate safety data sheet (SDS) is available upon request at the address: **info@id-solutions.fr**.

- **Infectious hazard**

- Wear protective clothing, including a lab coat, eye and face protection, and disposable gloves (synthetic latex-free gloves are recommended). Handle the kit reagents and the patient's samples in accordance with the required Good Laboratory Practices. Wash your hands thoroughly after completing the test.

- Do not smoke, drink, or eat in areas where samples or reagents of the kit are handled.

- Surfaces contaminated with a contaminating liquid must be thoroughly cleaned with an effective disinfectant. The material used for cleaning must be disposed of in a special container for contaminated waste.

- ⚠ Do not put solutions containing bleach in an autoclave.

- Dispose of all samples and material used to perform the test as if they were potentially infectious. The disposal of hazardous chemical and biological waste must be carried out in accordance with local regulations.

2. PRECAUTIONS OF USE

- The infectious and/or dangerous risks must be considered during handling, in accordance with the laboratory's current safety procedures.

⚠ **Patient samples and RNA samples stored in poor conditions may yield to erroneous results.**

- Do not use the kit or any of the kit reagents beyond the indicated expiration date.

- Do not mix reagents from other kits with different batch numbers.

- Do not use reagents that have been poorly preserved.

- All reagents should be thawed at +18 °C/+25 °C and homogenized before use. After thawing, handle the reagents on a cooling block at +2 °C/+8 °C or on ice and replace them as quickly as possible at -16 °C/-26 °C. The **ARM-IDSARSCOV2-N/E** reagent contains fluorophore-marked probes and must be protected from light especially during thawing.

Pay attention to all the points listed below:

- Follow the usage recommendations carefully to ensure that the test is performed correctly. Any discrepancies may affect test performance.

- Before use, check that the product and its components are not damaged, are compliant (number of tubes, volume), are properly labeled, and have been frozen upon receipt of the product.

- Use work areas separate from each other for the different testing stages: preparation of the samples, and preparation of amplification/detection reaction. It is recommended to adopt "a step forward" when completing the various stages. Wear gloves in each work area and change them before entering a different area.

- Use dedicated equipment and consumables for each work area and do not move them from one area to another.

- Avoid DNase and RNase contamination of the sample and kit components that could result in RNA/MATRIX DNA degradation, or PCR contamination by transfer, which could result in a false positive signal. Use nuclease-free laboratory equipment (pipettes, pipette tips, reaction vials) and wear gloves when performing the test. Use aerosol-resistant pipette tips for all pipetting steps.

- Separate and do not put positive and/or potentially positive material in contact with kit components.

- Do not open the tubes/reaction plates after amplification to avoid contamination by the amplicons.

- Do not reuse reaction tubes after a PCR (*e.g.* autoclaving tubes). Amplified nucleic acids will not be degraded.

5 PROTOCOL

1. PROTOCOL FOR THE EXTRACTION OF NUCLEIC ACIDS AND STORAGE CONDITIONS FOR RNA SAMPLES

For each extraction, include a negative extraction control (NEC-H2O) and a positive extraction control (see Test Principle).

Due to the risk of contamination by ribonucleases (RNases), RNA extraction is more difficult than DNA extraction. Strict precautions must be taken to avoid post-extraction RNA degradation. Refer to the extraction system manufacturer's recommendations for sample storage conditions after extraction.

2. REAL-TIME DETECTION PROTOCOL

- **Preparation of amplification reaction by RT-qPCR**

The sample to be amplified corresponds to the eluate product obtained after nucleic acid extraction.

Note: The amplification reaction must be performed in the area dedicated to amplification.

1. Prepare an analysis plan for the experimental samples and controls, taking care to keep the positive amplification control (**PAC-IDSARSCOV2-N/E**) away from samples. It is also advisable to frame the samples with two NAC controls, at the beginning and at the end of the sample series.
2. Thaw the **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit at a temperature of +18 °C/+25 °C and away from the light. Once thawed, keep them on a cooling block (+2 °C/+8 °C) or on ice.

⚠ **After use, place the reagents, ARM-SARSCOV-2-N/E and the PAC-IDSARSCOV2-N/E, as soon as possible at a temperature of -16 °C/-26 °C.**

3. Homogenize (vortex) the **ARM-IDSARSCOV2-N/E** tube and centrifuge them briefly.
4. Add **8 µL of ARM-IDSARSCOV2-N/E** per well.

⚠ **Please use equipment (96 wells plates or reaction tubes with adhesive films or caps for closure) suitable for the thermocycler to be used. Follow the manufacturer's instructions.**

⚠ **After adding the ARM, limit the time before the run on the thermocycler as much as possible.**

5. Add to the reaction mixture either:
   - 5 µL of extracted RNA, for each sample to be analyzed
   - 5 µL of PAC-IDSARSCOV2-N/E
   - 5 µL of NEC extract
   - 5 µL of nuclease free water (NAC)
6. Cover the plate or tubes with suitable adhesive film or caps.
7. Centrifuge for 1 minute at 1000 rpm to collect all the reagents.

⚠ **After adding the reagents, limit the time before the run on the thermocycler as much as possible.**

- **Programming the PCR amplification phase in the thermocycler**

Note: Please consult the manuals for the use of the different real-time PCR instruments for general information on their programming.

1. Program the reading of the following detectors on the thermocycler for each of the wells to be analyzed

TABLE 4. READING PARAMETERS

TARGET	READING CHANNEL	WAVELENGTH	QUENCHER
SARS-CoV-2	FAM™	530 nm	non fluorescent
SPIKE N501Y	VIC®/HEX™	560 nm	non fluorescent
SPIKE E484K	ROX / CAL Fluor® Red 610	610 nm	non fluorescent
Endogenous	Cy5	670 nm	non fluorescent

2. Enter the following amplification program on the thermocycler:

Table 5. RT- QPCR PROGRAM

STEPS		PROGRAM	CYCLES	ACQUISITION OF FLUORESCENCE			
				Applied Biosystems validated	Roche validated	CFX96	Agilent Aria MX
(1) Reverse Transcription		10 minutes 50 ° C	1	---	---	---	---
(2) Activation of Taq Polymerase		2 min 95 ° C	1	---	---	---	---
(3) Amplification	DNA denaturation	10 sec 95 ° C	40	---	---	---	---
	Hybridization and elongation	30 sec 60 ° C		FAM VIC ROX CY5	FAM (465 - 510) VIC / HEX (533 - 580) ROX (533-610) Cy5 (618-660)	FAM VIC ROX CY5	FAM VIC ROX CY5

Note: *Fluorescence reading is performed at the **end** of the elongation phase at 60°C.*

3. Select a final volume of **13 µL per PCR.**
4. Place the plate or tubes in the thermocycler and start the program.
5. Seal the plate for disposal in accordance with current local regulations.

6 RESULTS ANALYSIS AND INTERPRETATION OF RESULTS

Thanks to real-time PCR, the entire amplification kinetics is measurable (above the baseline) and may be quantified.

The fluorescence may then be expressed on a logarithm scale. The exponential phase is linearized.

Note: Ct = Threshold cycle, Cq = quantification cycle or CP = Crossing point

1. ANALYSIS OF THE RESULTS

- **Data analysis on validated Applied Biosystems instruments**

- Check that **None** has been selected in the **PASSIVE REFERENCE** field (the amplification mix contains a passive reference).

- SARS-CoV-2 target is analyzed once the FAM detector / reporter has been selected in the **DETECTOR/REPORTER** field.

- N501Y and E484K mutations are analyzed once the VIC and ROX detectors / reporters has been selected in the **DETECTOR/REPORTER** field.

- The endogenous target's analysis is performed once the **CY5** detector / reporter has been selected in the **DETECTOR/REPORTER** field.

- Let the software place the thresholds automatically.

- For each positive sample, a Ct is calculated in **FAM** and/or **VIC** and/or **ROX**. Negative samples marked as **UNDETERMINED** are displayed in the **Ct** column.

- For FAM negative samples, check the presence of a CY5 Ct.

- **Data analysis on validated Roche Instruments**

- Target analysis is carried out in **ABSOLUTE QUANTIFICATION** mode in **FAM, VIC and ROX**.

- Target analysis of the endogenous target is carried out in **ABSOLUTE QUANTIFICATION** mode in **Cy5**.

⚠ **Be sure to apply a previously calibrated four channel FAM™, VIC®/HEX™, ROX/CAL Fluor® Red 610 and Cyanine 5 compensation matrix specific to your LightCycler480 instrument.**

- For each positive sample of SARS-CoV-2, a **CROSSING POINT** (CP) is calculated in **FAM**.

- For each positive sample of N501Y and/or E484K mutations, a **CROSSING POINT** (CP) is calculated in **VIC** and/or **Rox**.

- For each negative sample, a **CROSSING POINT** (CP) is calculated in **Cy5**

Use the **FIT POINTS** method to determine the state (positive / negative) of the samples and controls.

- Analysis using the Fit Points method:

Analysis using the **FIT POINTS** method takes place in four stages: **COLOR COMPENSATION; CYCLE RANGE; NOISE BAND; ANALYSIS**.

In **STEP 1, apply the color compensation. To do so, in "Color Comp", select the compensation matrix in your database, once confirmed, check that the "Color Comp (Off)" button is displayed as "Color Comp (On)" to ensure proper application of the matrix.**

- For the analysis of the FAM channel: no compensation is required.
- For the analysis of the VIC channel: it is possible to apply compensation by selecting the FAM and VIC channels (FAM overflow into VIC).
- For the analysis of the Red610 channel: apply the compensation by selecting the VIC and Red610 channels (VIC overflow into Red610).
- For the analysis of the CY5 channel: no compensation is required.

In **STEP 2**, define the background noise range; for this, define the background between 2 and 16 cycles.

In **STEP 3** select the automatic definition of the background noise (noise band) and make sure that the horizontal line eliminates the background noise and crosses all the curves at the start of their exponential phase. Otherwise, adjust the threshold line manually.
Then, in **STEP 4**, select the automatic definition of the threshold.

- A CP in FAM and/or in VIC and/or in CAL Fluor® Red 610 is calculated for each positive sample. The negative samples are designated by the absence of a value in the **CP** column.

- For each negative sample in FAM, check the presence of a CP in CY5.

- **Data analysis on the CFX96 instrument**

- The targets are analyzed under the **QUANTITATION** tab, on the **FAM, VIC and CAL Fluor® Red 610** channels.

- The endogenous target is analyzed under the **QUANTIFICATION** tab, on the **CY5** channel.

- In the **SETTINGS** tab, select **Baseline Setting** then **Apply Fluorescence Drift Correction**

- In **SINGLE THRESHOLD** mode, manually move the threshold line so that it is:

• above the background noise

• in the exponential phase of each amplification curve, which generally corresponds to 5 to 10% of the final fluorescence of the sample.

- A Cq in FAM and/or in VIC and/or in CAL Fluor® Red 610 is calculated for each positive sample. The negative samples are designated by the **N/A** mention in the **Cq** column.

- For the negative samples in FAM, check the presence of a Cq in CY5.

- **Data analysis on the Aria MX instrument**

- Check that **None** has been selected in the field **REFERENCE DYE** of the **Plate Setup** menu (the amplification mix does not contain a passive reference

- In the **Plate Setup** select the **FAM, VIC, ROX** and **CY5** channel (Dye Name) and add the targets' name for each fluorophore (Target Name)

- The targets are analyzed in the **Analysis** menu in the **Analysis Criteria** tab (Cq visualization) and **Graphical Display** (curve display and threshold modification).

In the **Graphical Display** tab, move the threshold manually so that it is places itself:

- Over the background noise
- In the exponential phase of each amplification curve, which generally corresponds to 5 to 10% of the final fluorescence of the sample.

- A Cq in FAM and/or VIC and/or ROX is calculated for each positive sample. Negative samples or controls are designated by the indication **No Cq** in the column **Cq**.

- For negative samples in FAM and VIC, check the presence of a Cq in CY5.

2. VALIDATION AND INTERPRETATION OF RESULTS

- **Validation of the RT-qPCR test**

If one of the controls does not meet the validation criteria described in the table below, the assay is invalidated, and the results obtained for the sample should not be considered. The user can decide to repeat the assay after reviewing the procedure or may contact the manufacturer for assistance.

If the **positive amplification control (PAC)** is not positive for each target and/or Cq value is not within the Cq range specified in the batch related Quality Control Sheet (QCS), even if the negative control is valid, prepare new reactions using the remaining purified nucleic acids and a new PAC positive control.

If the **negative amplification control (NAC)** is not negative even though the positive control is valid, prepare new reactions using the remaining purified nucleic acids and a new NAC negative control.

If the necessary criteria are not fulfilled, the following items should be checked before repeating the test:
- Expiration of reagents used
- Functionality of the instrument used
- Correct performance of the test procedure

Table 6. VALIDATION CRITERIA FOR AN RT-qPCR ASSAY OF THE **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** KIT

CONTROL	EXPECTED RESULT	VALIDATION CRITERIA
PAC-IDSARSCOV2-N/E	Specific signal of SARS-CoV-2 detected in **FAM™**	Presence of the four characteristic graphic curves. Refer to the indicated Cq value of the related batch quality control sheet (QCS).
	Specific signal of the N501Y mutation detected in **VIC®/HEX™**	
	Specific signal of the E484K mutation detected in **ROX / CAL Fluor® Red 610**	
	Specific signal of the endogenous control detected in **Cy5**	
NAC	**No detection**	Total absence of a characteristic graphic curve

- **Result interpretation**

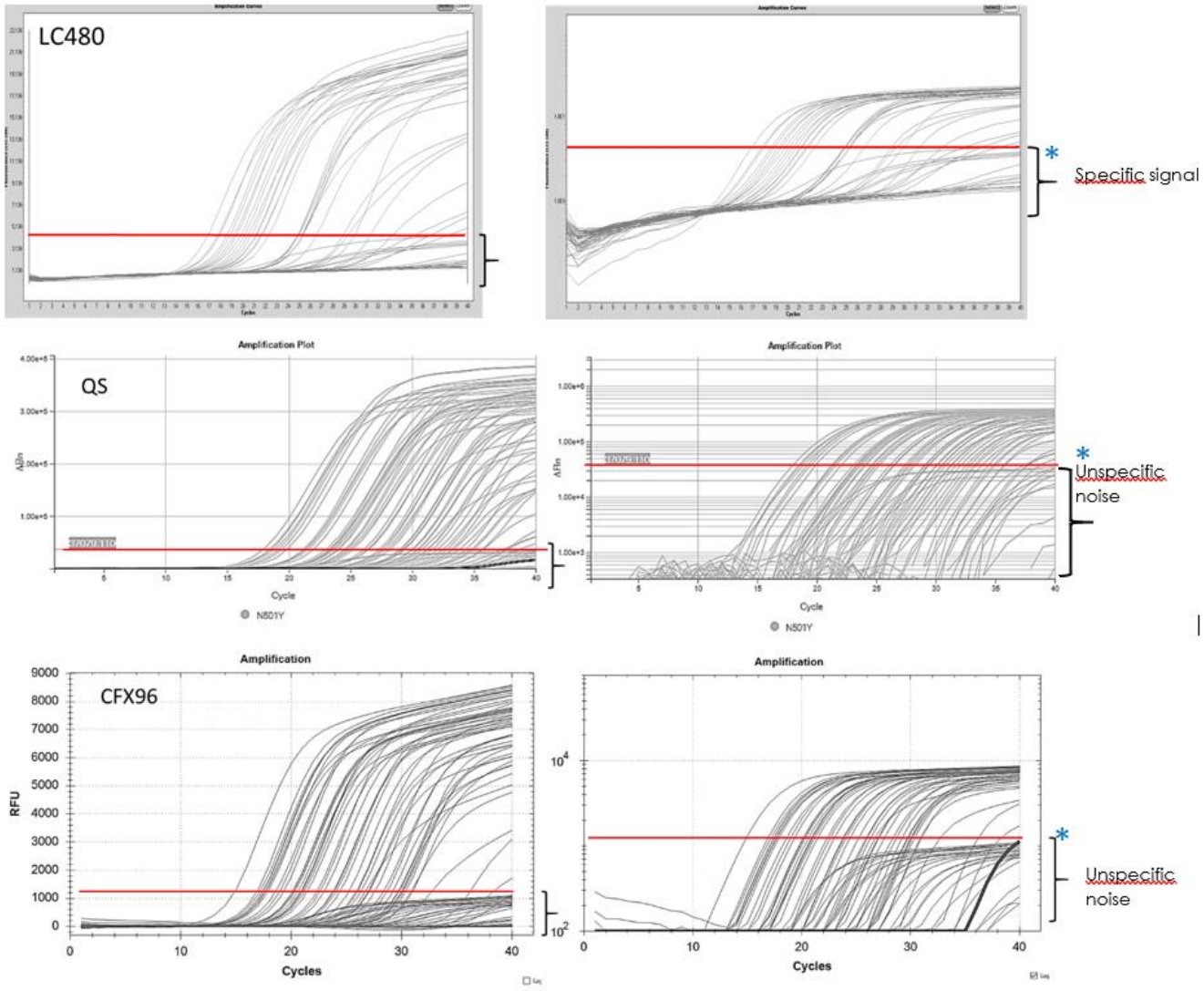
Presence or absence of SARS-CoV-2 RNA in the tested sample is **qualitatively** determined thanks to Cq (Quantification cycle) values obtained for each sample and for each target.

Note: Cq is also named Ct (Threshold cycle).

Adjust the threshold above any background noise signal towards the middle of the positive control PAC-IDSARSCOV2-N/E amplification curve (exponential phase) in logarithmic view. The chosen procedure to set the threshold must be used in a coherent way.

Due to the strong sequence homology between the variants (difference of a single nucleotide), the VIC channel (SPIKE N501Y mutation) can present a positive signal of low amplitude due to the non-specific hybridization of the probe on the not mutated N501 sequence.*

It is therefore important for the analysis of the VIC channel to set the threshold by following the examples described for each thermal cycler below:



Regarding FAM and Cy5 channels, follow the standard procedure of threshold assignment.

For each analyzed sample, the results must be interpreted according to the following criteria:

Table 7. RESULT INTERPRETATION

SARS-CoV-2 (FAM™)	SPIKE N501Y (VIC®/HEX™)	SPIKE E484K (ROX)	ENDO (Cy5)	INTERPRETATION	CONCLUSION
+	-	-	+ / -	Specific SARS-CoV-2 RNA detected	The sample contains detectable amounts of **SARS-CoV-2 specific RNA without detection of a mutation**.
+	+	-	+ / -	SARS-CoV-2 specific RNA carrying the **N501Y mutation**	The sample contains detectable amounts of **SARS-CoV-2 specific RNA carrying the N501Y mutation.**
+	-	+	+ / -	SARS-CoV-2 specific RNA carrying the **E484K mutation**	The sample contains detectable amounts of **SARS-CoV-2 specific RNA carrying the E484K mutation.**
+	+	+	+ / -	SARS-CoV-2 specific RNA carrying **N501Y and E484K mutations**	The sample contains detectable amounts of **SARS-CoV-2 specific RNA carrying the N501Y and E484K mutations.**
-	-	-	+	SARS-CoV-2 specific RNA **not detected**	The sample does not contain detectable amounts of SARS-CoV-2 specific RNA.
-	-	-	-	Non interpretable	Repeat the test or re-extract then repeat the test.

- The **presence** of a **characteristic curve and a Cq in FAM** and the **absence of a characteristic curve and a Cq in VIC/HEX and in ROX** corresponds to a **positive sample for SARS-CoV-2 without detection of a mutation.**
- The **presence** of a **characteristic curve and a Cq in FAM and in VIC/HEX** and the **absence of a characteristic curve and a Cq in ROX** corresponds to a **positive sample for SARS-CoV-2 with the N510Y mutation.**
- The **presence** of a **characteristic curve and a Cq in FAM and in ROX** and the **absence of a characteristic curve and a Cq in VIC/HEX** corresponds to a **positive sample for SARS-CoV-2 with the E484K mutation.**
- The **presence** of a **characteristic curve and a Cq in FAM, VIC/HEX and in ROX** corresponds to a **positive sample for SARS-CoV-2 with the N510Y/E484K mutations.**
- The absence of a **characteristic curve and a Cq in FAM, VIC/HEX and in ROX** paired with a **Cq presence for CY5** corresponds **to a negative sample for the SARS-CoV-2 target.**
- The **absence of any signal** makes the **result uninterpretable**.
- The absence of a **characteristic curve in FAM** and the **presence of a characteristic curve** and a Cq in **VIC/HEX** and/or **ROX** makes the result **uninterpretable.**
- A Cq superior or equal to 35 cycles for the SARS-CoV-2 target in FAM corresponds to a sample "below the limit of detection" and should not be considered for the search for mutations.

7 PERFOMANCES SUMMARY

The analytical performances of **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit were determined using *in silico* analysis and positive samples characterized by a reference method. All the following performances were obtained in accordance with the conditions described in the procedure and with the use of the following thermal cyclers:

Table 8. EVALUATED PERFORMANCES

Manufacturer	**Type**	**Evaluated performances**
Applied Biosystems	QuantStudio 5	Limit of detection
Roche	LightCycler 480 (System II)	Limit of detection
Bio-Rad	CFX96 Touch real-Time PCR detection System	Limit of detection
Agilent	AriaMx Real-time PCR	Limit of detection

1. ANALYTICAL SPECIFICITY

Inclusivity

The sequences targeted by the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** test are recommended and validated by the World Health Organization (**WHO**). These are specific sequences of the SARS-CoV-2 strain determined by the Centers for Disease Control and Prevention (CDC) in the U.S.A and the Charité Hospital in Berlin (Germany).

The specificity of the primers and probes allowing the detection of SARS-CoV-2 and SARS-COV-2 mutations detected by the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** test was determined by *in silico* analysis of the sequences on NCBI and GISAID databases. These analyzes show that the test specifically detects the SARS-CoV-2 strain, and the N501Y and E484K mutations and does not exhibit any cross-reactivity with other sequences in the database.

Exclusivity and biological interference

The specificity of the primers and probes allowing the detection of SARS-CoV-2 and of the SARS-COV-2 variants of the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** assay has been demonstrated through an analysis *in silico* and an experimental analysis carried out on a commercial panel containing pathogens likely to be found in patient samples (see list below). The extraction was performed on the IDEAL® 32 instrument with the IDGENE MAGVIRUS250 kit (ID SOLUTIONS) and the amplification on the QuantStudio 5 Applied Biosystems thermocycler.

The pathogens were tested:

- Without co-infection with SARS-CoV-2 with or without the del69-70 and N501Y mutations (exclusivity study).

Results: No cross-reaction or interference was observed for the following pathogens:

- **Viruses**: human coronavirus: 229E, NL63, OC43, HKU1, SARS and MERS-CoV, adenovirus (type 1; 3; 31), human metapneumovirus (MPVh), parainfluenza virus type 1 to 4, influenza A, influenza B, respiratory syncytial virus (RSV), and rhinovirus.
- **Bacteria**: *Chlamydia pneumoniae, Bordetella pertussis, Bordetella parapertussis, Mycoplasma pneumoniae*.

- Co-infected with SARS-CoV-2 with or without the del69-70 and N501Y mutations (study of biological interferences).

In order to measure the interference on the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit, several mixtures (listed below) were made and tested in order to mimic strongly or weakly positive samples for the targets of interest in the presence of pathogens:

- The sample named "**RP**" corresponds to the mixture of all the respiratory pathogens mentioned above with the exception of the SARS-CoV-2 virus and its variants.

- The sample named "**RP + SARS-CoV-2**" corresponds to the mixture of all the respiratory pathogens mentioned above, including **SARS-CoV-2 "VOC202012 / 01**" in mid concentration.

- The sample named "**RP + variant 501Y.V1**" corresponds to the mixture of all the respiratory pathogens mentioned above, including the **501Y.V1 virus in mid concentration**.

- The sample named "**RP + variant 501Y.V2**" corresponds to the mixture of all the respiratory pathogens mentioned above except for the SARS-CoV-2 virus but including the **501Y.V2 virus in mid concentration**.

The table below expresses the results obtained for the triplicate passage of the mixtures considered.

Table 9. RESULTS OF INTERFERENCE STUDY

	SARS-CoV-2	SPIKE N501Y	SPIKE Del69-70
RP	-	-	-
RP + SARS-CoV-2	+	-	-
RP + variant 501Y.V1	+	+	+
RP + variant 501Y.V2	+	+	-

Results: No interference observed for pathogens listed above.

2. ANALYTICAL SENSITIVITY - LIMIT OF DETECTION

The analytical sensitivity (or 95% detection limit) of the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex kit** was determined on two ranges of 5 dilutions made from an extract of RNA from the inactivated viral suspension supplied. by the CNR of Lyon corresponding to the line 20H / 501Y.V2 (Variant South Africa). These samples were previously titrated by digital PCR (internal method ID SOLUTIONS). These samples were then amplified with the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex kit** on the validated thermal cyclers. The LoD was determined as the lowest concentration of template that can be reliably detected with a confidence level of 95%.
Each sample was tested 24 times *.

The tables below express the results on the average of the 24 replicates for each of the targets.

Tableau 10. RESULTS FOR THE ANALYTICAL SENSITIVITY FOR SARS-COV-2 TARGET (*TESTED ON 8 REPLICATES ONLY)

Strain	Targets	Thermocycler	Concentration (copies/PCR)	Average Cq	Standard deviation	CV (%)	% Detected
20H/501Y.V2 "South African variant"	**SARS-CoV-2 (N2 + RdRP)**	QuantStudio 5™ Applied Biosystems	80*	32,10	0,21	0,7%	100,00%
			40*	32,97	0,45	1,4%	100,00%
			20	34,34	0,64	1,9%	100,00%
			10	**35,45**	**0,77**	**2,2%**	**100,00%**
			5	35,23	4,75	13,5%	92,00%
		CFX96 Touch real-Time PCR detection System	80*	33,01	0,29	0,9%	100,00%
			40*	33,70	0,42	1,2%	100,00%
			20	34,89	0,39	1,1%	100,00%
			10	**35,96**	**0,81**	**2,2%**	**100,00%**
			5	36,87	1,01	2,7	92,00%
		LightCycler 480 (SystemII)	80*	31,81	0,55	1,7%	100,00%
			40*	32,23	0,55	1,7%	100,00%
			20	33,90	0,59	1,7%	100,00%
			10	**34,25**	**0,63**	**1,8%**	**100,00%**
			5	35,99	0,67	1,9%	83,00%
		Aria MX	80*	32,06	1,82	5,7%	100,00%
			40*	34,16	0,69	2,0%	100,00%
			20	35,47	0,69	1,9%	100,00%
			10	36,53	0,71	1,9%	100,00%
			5	**37,36**	**0,88**	**2,3%**	**100,00%**

Table 11. RESULTS FOR THE ANALYTICAL SENSITIVITY FOR THE SPIKE N501Y TARGET (*TESTED ON 8 REPLICATES ONLY)

Strain	Targets	Thermocycler	Concentration (copies/PCR)	Average Cq	Standard deviation	CV (%)	% Detected
20H/501Y.V2 "South African variant"	**SPIKE N501Y**	QuantStudio 5™ Applied Biosystems	20*	35,44	0,50	1,4%	100,00%
			10	**36,11**	**0,82**	**2,3%**	**100,00%**
			5	37,36	0,60	1,6%	88,00%
			2.5	37,91	0,67	1,8%	67,00%
		CFX96 Touch real-Time PCR detection System	20*	34,27	0,53	1,5%	100,00%
			10	34,65	0,70	2,0%	100,00%
			5	**35,97**	**0,75**	**2,1%**	**96,00%**
			2.5	36,97	0,78	2,1%	67,00%
		LightCycler 480 (SystemII)	20*	34,08	0,47	1,4%	100,00%
			10	**34,14**	**0,83**	**2,4%**	**100,00%**
			5	35,98	0,59	1,6%	79,00%
			2.5	35,98	0,46	1,3%	29,00%
		Aria MX	20*	31,89	3,12	9,8%	100,00%
			10	**34,68**	**1,26**	**3,6%**	**100,00%**
			5	36,14	0,66	1,8%	83,00%
			2.5	36,71	0,88	2,4%	67,00%

Table 12. RESULTS FOR THE ANALYTICAL SENSITIVITY FOR THE SPIKE E484K TARGET (*TESTED ON 8 REPLICATES ONLY)

Strain	Targets	Thermocycler	Concentration (copies/PCR)	Average Cq	Standard deviation	CV (%)	% Detected
20H/501Y.V2 "South African variant"	**SPIKE E484K**	QuantStudio 5™ Applied Biosystems	20*	35,97	0,57	1,6%	100,00%
			10	**36,47**	**0,85**	**2,3%**	**100,00%**
			5	37,53	0,65	1,7%	88,00%
			2.5	38,05	0,78	2,1%	67,00%
		CFX96 Touch real-Time PCR detection System	20*	35,04	0,74	2,4%	100,00%
			10	35,15	0,84	2,4%	100,00%
			5	**36,68**	**0,97**	**2,6**	**96,00%**
			2.5	37,52	0,89	2,4%	63,00%
		LightCycler 480 (SystemII)	20*	35,75	0,51	1,4%	100,00%
			10	**35,54**	**0,97**	**2,7%**	**100,00%**
			5	37,08	0,48	1,3%	67,00%
			2.5	36,85	0,85	2,3%	33,00%
		Aria MX	20*	30,27	5,00	16,5%	100,00%
			10	**34,63**	**0,94**	**2,7%**	**100,00%**
			5	36,11	0,74	2,1%	83,00%
			2.5	36,82	1,10	3,0%	67,00%

The limit of detection (LoD 95%) of the **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** kit is **10 copies / PCR** for **SARS-CoV-2** and **for N501Y and E484K mutations**.

3. PRECISION

Precision studies include repeatability (intra-assay) and intermediate precision (inter--assays) studies. The accuracy of the test was evaluated on different dilutions of the positive amplification control (PAC-IDSARSCOV2-N/E). These non-native samples were then amplified with the **ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex kit** .

- The intra-assay precision (Table 12) was evaluated on 16 repetitions of 3 dilutions of a range of synthetic DNA from each separate target at concentrations> 3x the LoD as well as on 3 dilutions of PAC- IDSARSCOV2- N / E Lot p0121-01.
- The inter- assay studies (Tables 13 and 14) were carried out on the same batch of reagents tested on 3 different instruments (2 QuantStudio 5, CFX96 Touch real-Time PCR detection System) by 1 user on 3 independent test series ; with 8 repetitions per sample, on 3 samples, including 2 samples with concentrations close to the LoD.

The mean Cq, standard deviation, intra-assay and inter-assay precision (% CV) for each of the samples in the panel are presented in the following tables:

Table 13. REPEATABILITY OF THE TEST

Targets		Concentration (copies / PCR)	Repeatability		
			Medium cq	Standard deviation	% CV
SARS-CoV-2		500	28.88	0.20	0.71%
		100	31.89	0.45	1.40%
		50	32.63	0.24	0.74%
SPIKE N501Y		500	28.01	0.16	0.56%
		100	30.24	0.23	0.77%
		50	31.37	0.25	0.80%
SPIKE E484K		500	28.76	0.34	1.19%
		100	30.57	0.46	1.52%
		50	31.73	0.44	1.38%
PAC-IDSARSCOV2- N / E	SARS-CoV-2	Pure	29.65	0.10	0.35%
	SPIKE N501Y	Pure	26.52	0.08	0.29%
	SPIKE E484K	Pure	26.58	0.17	0.63%
	SARS-CoV-2	1/5	32.15	0.12	0.36%
	SPIKE N501Y	1/5	28.96	0.10	0.64%
	SPIKE E484K	1/5	28.87	0.17	0.60%
	SARS-CoV-2	1/10	33.19	0.21	0.64%
	SPIKE N501Y	1/10	29.98	0.11	0.38%
	SPIKE E484K	1/10	30.01	0.14	0.47%
	Endogenous	1/10	31,65	0,27	0,84%

Table 14. INTERMEDIATE RELIABILITY OF THE TEST ON EACH OF THE TARGETS

Targets	Level (copies / PCR)	Run 1 (QuantStudio 5 n°1)		Run 2 (QuantStudio 5 n°2)		Run 3 (CFX96)		Runs 1+2+3		
		Average Cq	Standard deviation	Average Cq	Standard deviation	Average Cq	Standard deviation	Average Cq	Standard deviation	Average Cq
SARS-CoV-2	500	29.96	0.10	29.66	0.09	29.59	0.15	29.74	0.20	0.66%
	100	32.23	0.15	32.12	0.15	31.68	0.21	32.01	0.29	0.91%
	50	33.24	0.23	33.18	0.27	32.69	0.27	33.04	0.31	0.92%
SPIKE N501Y	500	28.91	0.10	29.13	0.19	29.28	0.07	29.11	0.18	0.63%
	100	31.39	0.13	31.54	0.19	31.67	0.14	31.53	0.14	0.44%
	50	32.58	0.28	32.46	0.23	32.63	0.22	32.56	0.09	0.27%
SPIKE E484K	500	29.56	0.22	29.13	0.33	28.52	0.20	29.07	0.52	1.80%
	100	31.99	0.09	31.40	0.18	30.97	0.31	31.45	0.52	1.64%
	50	33.17	0.33	32.37	0.21	31.97	0.19	32.50	0.61	1.88%

Table 15. INTER-ASSAYS STUDY ON PAC-IDSARSCOV2-N/E

		PAC-IDSARSCOV2-N/E Target			
		SARS-COV-2	SPIKE N501Y	SPIKE E484K	Endogenous
Run #1 (QuantStudio 5 n°1)	Average Cq	30,46	26,38	26,98	29,73
	Standard deviation	0,07	0,01	0,13	0,17
Run #2 (QuantStudio 5 n°2)	Average Cq	30,54	26,48	26,23	30,03
	Standard deviation	0,12	0,29	0,36	0,40
Run #3 (CFX96 Touch real-Time PCR detection System)	Average Cq	29,74	26,52	25,27	29,95
	Standard deviation	0,06	0,03	0,16	0,11

PAC-IDSARSCOV2-N/E	Level	ESSAI 1+2+3

Target		Average Cq	Standard deviation	%CV
SARS-CoV-2	**Not diluted**	30,25	0,40	1,32%
SPIKE N501Y	**Not diluted**	26,46	0,15	0,55%
SPIKE E484K	**Not diluted**	26,16	0,79	3,02%
Endogène	**Not diluted**	29,90	0,24	0,82%

8 EVALUATION OF CLINICAL PERFORMANCES

The clinical evaluation of the **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** assay used for the detection of SARS-CoV-2 coronavirus variants by real-time RT-PCR was performed by a retrospective study, totaling 209 nucleic acid extracts characterized for SARS-CoV-2 by a CE-Marked RT-PCR method considered as predicate method.

These extracts were obtained from native samples of patients collected in the context of suspected SARS-CoV-2 respiratory infection.

Samples that were positive in the first-linr were retested in the second-line with IDSARSCOV-N/E and sequenced (NGS technique) in parallel by a laboratory certified by the National Reference Center for Respiratory Infections (including influenza).

The results obtained with IDSARSCOV-N/E are compared to the commercial first-line predicate test for concordance of detection of SARS-CoV-2 as well as to the sequencing method that serves as the reference method for concordance of detection of N501Y and E484K targeted mutations.

Table 16. Relative agreement for SARS-CoV-2 detection

Predicate *CE-marked RT-PCR assay*	**IDSARSCOV-N/E**		
	Negative	Positive	**Total**
Negative	108	--	108
Positive	--	101	101
Total	108	101	209

Overall agreement of SARS-CoV-2 detection **100%** [98.6;100.0] IC (95%)

Table 17. N501Y MUTATION DETECTION

Reference Next Generation sequencing	**IDSARSCOV-N/E**		
	Negative	Positive	**Total**
Negative	12	-	**12**
Positive	-	82	**82**
Total	**12**	**82**	**94**

Positive agreement of N501Y detection	82/82	**100%** [93.3;100.0] IC (95%)
Relative agreement of N501Y detection	94/94	**100%** [96.8;100.0] IC (95%)

Table 18. E484K MUTATION DETECTION

Reference Next Generation sequencing	IDSARSCOV-N/E		
	Negative	Positive	**Total**
Negative	31	--	**31**
Positive	1*	61	**62**
Total	**32**	**61**	**93**

A sample appears to be discreptant with the reference method. Its profile shows an inhibition of RT-PCR resction on the ID ™ SARS-CoV-2 / N501Y / E484K Quadruplex kit, which explains the loss of detection of the E484K mutation.

Positive agreement of E484K detection	61/62	**100%** [95.3;100.0] IC (95%)
Relative agreement of E484K detection	92/93	**100%** [96.8;100.0] IC (95%)

Conclusion

The results obtained with the kit **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** are in perfect agreement with the reference RT-PCR method, CE-marked, used routinely for the characterization of samples extracted from patients with suspected SARS-CoV-2 infection.

The results obtained with the kit **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** agree with the sequencing method used as reference for the detection of the N501Y mutation.

The results obtained with the kit **ID™ SARS-CoV-2 / N501Y / E484K Quadruplex** agree with the sequencing method used as reference for the detection of the E484K mutation.

9 TROUBLESHOOTING

INVALID ASSAYS

Potential cause	Solution
The characteristic amplification curves do not appear due to the degradation of the reagents in the kit, including the amplification mix.	Check that the storage conditions and handling of the reagents are scrupulously followed (see section 3). Check reagent expiration dates.
Error in the implementation of the test protocol.	Check the entire test protocol followed against the protocol described in these instructions for use
Contamination of reagents.	Check the conditions of storage and conditions of use of reagents. Validate the absence of contamination of the reagents. Decontaminate equipment and premises.

PATHOGEN PRESENT BUT NOT DETECTED IN SAMPLES: FALSE NEGATIVES

Potential cause	Solution
Degradation of the reagents in the kit, including the amplification mix.	Check that the conditions of storage and handling of the reagents are scrupulously followed (see section 3). Check reagent expiration dates Check that the validation criteria for controls are met.
Problems collecting, transporting, or storing of the sample	Check that the recommendations of the laboratory and / or the manufacturer of the sampling equipment have been observed. Check the time between the sampling and the analysis.
Problem encountered during the extraction.	Check that the extraction conditions and maintenance operation of the extraction devices have been respected in accordance with the manufacturer's protocol. Validate the results of the extraction control (if it was carried out as recommended). Check that the samples have been homogenized before starting the extraction step.
Error in the implementation	Check the entire test protocol followed against the protocol described in these instructions for use.
Programming error	Check all the programming parameters of the thermocycler as recommended in these instructions for use. Contact **info@id-solutions.fr** for more details.
Problem during the amplification step	Validate thermocycler compliance.

	Validate all the elements implemented in the reaction (consumables used, properly sealed plate, etc.). Refer to the equipment manufacturer's manual for more details.
Problem of results analysis	Validate the positioning of the positivity threshold Contact **info@id-solutions.fr** for more details.
Problem interpreting the results	Validate that all validation criteria are met.

PATHOGEN NOT PRESENT BUT DETECTED IN SAMPLES: FALSE POSITIVE

Potential cause	Solution
Contamination	Check the conditions of storage and use of reagents. Check that the validation criteria for controls are met. Validate the absence of contamination of the reagents. Decontaminate equipment and premises.
Error in the implementation	Check the entire test protocol against the protocol described in these instructions for use.
Programming error	Check all of the thermocycler's programming parameters as recommended in these instructions for use. Contact **info@id-solutions.fr** for more details.
Problem of analysis of results	Validate the positioning of the positivity threshold Contact **info@id-solutions.fr** for more details.
Problem interpreting the results	Validate that all validation criteria are met.

INHIBITION OF SAMPLES

Potential cause	Solution
Problem encountered during the extraction.	Check that the extraction conditions have been respected in accordance with the manufacturer's protocol. Validate the results of the extraction control (if it was carried out as recommended). Check the validity of the batches of reagent used. Check that the samples have been homogenized before starting the extraction step.

10 LIMITATIONS

- The product is intended to be used only by healthcare professionals who have received instructions and training in real-time PCR techniques related to the thermocycler used and *in vitro* diagnostic procedures.
- Respect good laboratory practices to guarantee the proper functioning of this test. Preserve the purity of the kit components when preparing the samples, which is a source of contamination. Laboratory and reagents must be monitored to prevent them from being contaminated or containing impurities. If a reagent is suspected, the laboratory should proceed with its disposal.
- Do not use this test directly on the patient sample, an adequate nucleic acid extraction method must be used before performing the test. It is important to assess the quality of the extraction step before testing.
- The reagents provided with the kit are ready to use, so no dilution should be made as performance would be affected.
- If the sample or reagents are not used according to the instructions for use, the test result may be incorrect. The repetition of the test in a new sample from the same patient should be considered if Covid-19 is suspected or if an error has occurred during the procedure.
- The procedure and interpretation of the results described for the test must be followed when performing RNA detection in the samples. It is recommended that the user of the kit read the instructions for use carefully before carrying out the test. The test procedure must be strictly followed.
- Obtaining a negative result on samples does not exclude infection with SARS-CoV-2 or associated variants.
- Positive results do not exclude bacterial infection or co-infection with other viruses.
- The procedures for collecting, transporting, storing and processing patient samples must be scrupulously observed according to the recommendations given by the manufacturer of systems or kits for the extraction of nucleic acids, in order to ensure optimal performance of the test.
- Potential mutations in the targeted areas of the virus genome recognized by the primers and / or the probes of the test can prevent its detection.

11 TECHNICAL SUPPORT AND DOCUMENTATION

For any questions or technical support, please contact us at the following address: **info@id-solutions.fr.**

12 SYMBOLS USED

The following symbols may appear on the packaging and labelling:

	Contains enough reagents for n reactions
	Batch number
	Reference number
	Positive Control
	Expiration date
	Refer to the instruction for use before any use
	Manufacturer
	Keep away from the sun
	Temperature limit
	Warning

13 LAST REVISION

VERSION	EDITING DATE	REFERENCES	DESCRIPTION OF THE MODIFICATION
ver0321-1	03/30/2021	DOC0842	Translation from French revision ver0321-1