HANDBOOK

Bio-T kit® SARS-CoV-2 UK & N501Y Variants

Cat. N° BIOTK125 - 500 reactions

Identification of 20I/501.Y (UK), 20H/501Y.V2 (South African) and 20J/501Y.V3 (Brazilian) variants of SARS-CoV-2 by real-time RT-PCR (qRT-PCR) with Exogenous internal positive control (IPC)

HUMAN

Sample types

- Oral, nasal swabs
- Samples of the lower respiratory tract (Alveolar bronchial washing, sputum)

Recommended nucleic acids (NA) extractions

- Magnetic beads extraction (ex: BioSellal – BioExtract® SuperBall® Cat. N° BES384, BioSellal – BioExtract® Premium Mag Cat. N° BEPM96, BEPM1K, BEPM2K, BEPM5K)
- Silica membrane columns extraction (ex : BioSellal – BioExtract® Column Cat. N° BEC050 or BEC250)

Research use only

ADMINISTRATIVE INFORMATION

Name and address of the producer:

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Manufacturing, Control and Packaging location:

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DOCUMENTS MANAGEMENT

The Bio-T kit® SARS-CoV-2 UK & N501Y Variants has two technical handbooks:

- The extraction handbook shared between the Bio-T kit® SARS-CoV-2 UK & N501Y Variants , Bio-T kit® TriStar Covid-19 and Bio-T kit® 4Plex Covid & Flu displaying BioSellal's recommended extraction protocols for each type of sample.
- The Bio-T kit® SARS-CoV-2 UK & N501Y Variants qRT-PCR handbook, presenting the instruction information to perform the qRT-PCR.

The last versions in use for each handbook are indicated on the certificate of analysis (CA) provided with the Bio-T kit® SARS-CoV-2 UK & N501Y Variants.

Besides these two handbooks, a performances confirmation handbook is available on request, contact BioSellal (contact@biosellal.com).

MODIFICATIONS MANAGEMENT

BioSellal indicates modifications done to this document by highlighting them using the rules presented in the Table below:

MODIFICATIONS MANAGEMENT			
Type of modification Highlighting color	**Minor modifications**	**Type 1 Major modifications**	**Type 2 Major modifications**
Impact on revision / version	**Change of revision date** **No change of version**	**Change of revision date** **+ change of version**	**Change of revision date** **+ change of version**
Examples of modifications	Corrections: typographical, grammatical or turns of phrase	EPC reference modification	Modification of Master Mix composition
	Addition of new sample type for extraction	Exogenous IPC reference modification	Modification of validated extraction protocol
	Addition of information giving more details or alternative protocol		
	Addition/Suppression of optional information		

PRESENTATION

Recommendations for sampling, shipping and storage of samples

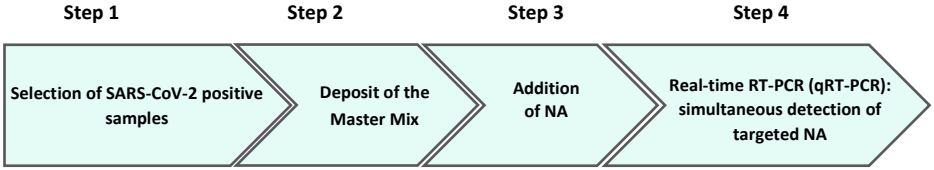
The Bio-T kit® SARS-CoV-2 UK & N501Y Variants is a second line test allowing the identification of 20I/501Y.V1 (UK), 20H/501Y.V2 (South African) and 20J/501Y.V3 (Brazilian) variants of SARS-CoV-2 from nucleic acids previously extracted and found positive for SARS-CoV-2 using a kit such as Bio-T Kit® TriStar Covid-19 or equivalent kit allowing the SARS-CoV-2 detection.
For further recommendations on sampling, shipping and storage of samples, please refer to the Bio-T kit® TriStar Covid-19 handbook.

Description of the Bio-T kit® SARS-CoV-2 UK & N501Y Variants

The **Bio-T kit® SARS-CoV-2 UK & N501Y Variants** (Cat. N° BIOTK125) contains a ready to use **one-step RT-PCR Master Mix** allowing the detection **in the same reaction well of**:

- ***E gene* of all Sarbecovirus including SARS-CoV-2** with a TEXAS RED labelling,
- **N501Y *S gene* mutation** (shared by several variants including 20I/501Y.V1 (UK), 20H/501Y.V2 (South-African) and 20J/501Y.V3 (Brazilian) variants of SARS-CoV-2) with a VIC labelling,
- **Deletion Δ69-70 of *gene S*** specific of 20I/501Y.V1 (UK) and mink cluster V (ΔFVI-spike, Denmark) variants with a FAM labelling,
- **An Exogenous internal positive control IPC RNA**, with a Cy5 labelling. It validates the proper conservation of nucleic acids (no degradation) and the absence of inhibition. As a reminder, exogenous IPC is added during the extraction of nucleic acids.

Description of the whole process



qRT-PCR handbook of the Bio-T kit® SARS-CoV-2 UK & N501Y Variants		
Ready-to-use Master Mix MMUKN501Y-A	Samples NC/NCS Process positive control EPC (EPCUKN501Y-B)	Dyes: FAM/VIC/TEXAS RED/Cy5 Passive reference: None Program: UK & N501Y variants Standard ramping

Kit content and storage

Table 1. Description of the kit contents

Description	Reference	Volume/tube	Presentation	Storage
Master Mix (MM) Ready to use	MMUKN501Y-A	7500 µl	Grey cap tube Bag A	≤-16°C Protected from light, « MIX » Area
Exogenous Internal Positive Control (IPC)	IPCTRISTAR-B	2500 µl	Grey cap tube Bag B	≤-16°C « Extraction » Area
External Positive Control (EPC) SARS-CoV-2 positive PCR control including N501Y mutation and SΔ69-70 deletion	EPCUKN501Y-B	200 µl	Red cap tube Bag C	≤-16°C « Addition of Nucleic acids » Area
Water RNase/DNase free	Aqua-A	1 ml	Blue cap tube Bag C	5°C ± 3 or ≤-16°C « Addition of Nucleic acids » Area

Kit reagents are stable until the expiration date stated on the label, subject to compliance with good storage conditions.

List of consumables and reagents not included in kit

Table 2. Consumables and reagents not included in kit				
Consumables/ Reagents	**Description**		**Provider**	**Cat. N°**
ATL Buffer	Lysis Buffer		BioSellal	ATL19076
BioExtract® Column	DNA/RNA column extraction kit (50)		BioSellal	BEC050
BioExtract® Column	DNA/RNA column extraction kit (250)		BioSellal	BEC250
BioExtract® SuperBall®	DNA/RNA Magnetic beads extraction kit (4 x 96)		BioSellal	BES384
BioExtract® Premium Mag	DNA/RNA Magnetic beads extraction kit	96	BioSellal	BEPM96
		1000		BEPM1K
		5000		BEPM5K

For consumables related to the thermal cycler, refer to the user manual of the device.

List of reagents to confirm laboratory performances

Synthetic RNA of *E gene* of SARS-CoV-2 and mutated *S gene* (titrated in number of copies/RT-PCR) used by BioSellal for the validation could be provided. BioSellal sells these reagents under the following references:

Table 3. Optional reagents*			
Reagent	**Description**	**Provider**	**Cat. N°**
***E gene* of SARS-CoV-2 RNA**	Quantified RNA of SARS-CoV-2	BioSellal	cARN-ESRAS-001
Mutated *S gene* of SARS-CoV-2 RNA	Quantified RNA of SARS-CoV-2	BioSellal	cARN-UKSRAS-001

* These reagents are available only on demand, please contact BioSellal (contact@biosellal.com).

Main critical points

- Wear appropriate personal protective equipment (lab coat, disposable gloves frequently changed).
- Work in dedicated and separate areas to avoid contamination: "Extraction" (unextracted samples storage, extraction equipment area), "Mix" (ready to use Master Mix storage, qRT-PCR plates preparation), "Nucleic acids Addition" (Nucleic Acids storage and addition of extracted nucleic acids and controls in the qRT-PCR plate), "PCR" (final area containing the thermal cycler(s)).
- Use dedicated equipment for each working area (gloves, lab coat, pipettes, vortex, ...).
- Use filter tips.
- Before use, thaw all components at room temperature.
- **One-step RT-PCR Master-Mix is less stable than PCR Master-Mix. To guarantee its optimal performance, it is mandatory to extemporaneously defrost the tubes just before the use, to vortex it, to keep it at 5°C ± 3 during the deposit and to refreeze it immediately afterwards.**
- Vortex and spin briefly (mini-centrifuge) all reagents before use.
- Avoid the repetition of freezing-thawing cycles for samples, lysates, extracted nucleic acids.
- **SARS-CoV-2 genome consists of RNA. Working with RNA is more demanding than working with DNA** (RNA instability and omnipresence of the RNases). For these reasons, special precautions must be taken:
    - Always wear gloves, change them frequently, especially after contact with skin or working surfaces.
    - Treat all surfaces and equipment with RNases inactivation agents (available commercially).
    - When wearing gloves and after material decontamination, minimize the contact with surfaces and equipment in order to avoid the reintroduction of RNases.
    - Use “RNase free” consumable.
    - It is recommended to store the RNA at ≤ 5°C ± 3 during the manipulation and then freeze it as soon as possible, preferably at ≤ -65°C or by default at ≤ -16°C.
    - Open and close tubes one by one in order to limit the opening times and avoid any contact with RNases present in the environment (skin, dust, working surfaces…).

IDENTIFICATION OF 20L/501.Y, 20H/501Y.V2 AND 20J/501Y.V3 VARIANTS OF SARS-COV-2 BY qRT-PCR WITH BIOTK125

Global Procedure

1) **Establish qRT-PCR plate setup** defining each sample position and **including the following controls**:

- **Negative Control Sample (NCS)**: water (or PBS) replaces the sample from the first step of sample preparation.
  This control is mandatory for each extraction series. It is however optional for the Bio T Kit® SARS-CoV-2 UK & N501Y Variants since the absence of contamination during extraction has been verified during the screening step.

- **Negative Amplification Control (NC)**: 5 µl of water RNase/DNase free (Aqua-A tube, **blue** cap) replaces sample Nucleic Acids extract on qRT-PCR plate.
  This control is mandatory in absence of NCS

- **External Positive Control (EPC)**: Synthetic DNA provided (tube **EPCUKN501Y-B**, **red** cap), containing specific target of SARS-CoV-2 including N501Y mutation and SΔ69-70 deletion of *S gene*.
  This control is mandatory unless a positive process control is used.

⚠ CAUTION: *Due to the high sensitivity of the real-time RT-PCR process, good laboratory practice is essential for the proper performance of this test. Therefore, care must be taken to ensure that reagents are not contaminated. This includes opening and handling the Positive Control (EPC) in a delimited area away from other components and taking precautions to avoid cross-contamination with other sample during plate deposition. The NC and NCS Negative Controls ensure respectively that the Master Mix and the overall process including extraction are free of contamination. If these controls are positive, refer to the table on page 13.*

- If available, **a Process Positive Control (MRI),** a weak positive sample for each target is extracted in parallel with tested samples. After qRT-PCR, MRI Ct values will be monitored on a Shewhart control card. Obtaining conform Ct values validates the whole process. In this case, the use of the EPC, provided with the kit, is not mandatory.

2) qRT-PCR plate preparation

In the "MIX" dedicated area

1. After thawing, vortex and rapid centrifugation, **transfer 15 µl Master Mix MMUKN501Y-A** (grey cap) in each well of interest (samples and controls).
   ⚠ *NOTE: One-step RT-PCR Master-Mix is less stable than PCR Master-Mix. To guarantee its optimal performance, it is mandatory to extemporaneously defrost the tubes just before the use, to vortex it, to keep it at 5°C ± 3 during the deposit and to refreeze it immediately afterwards.*

In the "Nucleic Acids addition" dedicated area

2. **Add 5 µl of extracted nucleic acids (or NCS, water, MRI or EPC**: **EPCUKN501Y-B red** cap tube) in each well of interest. Make sure to pipet out in the bottom of the well, in the Master Mix, and to avoid the formation of bubbles.

Note: if the exogenous IPC was not added during sample extraction, it can be added directly in the qRT-PCR plate:
- Add 1 µl of IPC (grey cap) with the extracted nucleic acids
- Or directly add the IPC (1 µl per reaction) in an aliquot of Master Mix before the deposits of 16 µl of this mix into each well of interest. Then add 5 µl of extracted nucleic acids.
The reaction volume will be increased to 21 µl, without impacting the performances of the qRT-PCR.

3. **Seal the plate with an optically clear sealer** or **close the strip caps**.

In the "PCR" amplification dedicated area

4. **Define the thermal cycler parameters** (see Table 4, Table 5, Table 6)

5. It is recommended to **spin the plate down prior to place it in the thermal cycler**, to prevent drops in the well pit walls.

6. Start the qRT-PCR program. Approximate run time: 90min.

3) Thermal cycler settings

This kit was developed and validated on AriaMx™ (Agilent Technologies, Fast ramping by default) and confirmed on ABI PRISM® 7500 Fast (Applied Biosystems) in standard ramping and QuantStudio 5 Real Time PCR system (Applied Biosystems). It is compatible with all thermal cyclers with at least 6-FAM, VIC, TEXAS RED and Cy5 channels. For more information, contact our technical support.

Table 4. Thermal cycler configuration		
	ABI PRISM® 7500 Fast / QuantStudio 5	**AriaMx™**
Mode	Quantitation – Standard curve	Quantitative PCR, Fluorescence Probe
Ramping	Standard Ramping	Ramping Fast by default
Passive Reference	**NONE**	**NONE**

Table 5. Thermal cycler Settings			
Target	**Detectors**		**Final Volume / well**
	Reporter	**Quencher**	
***E gene* of SARS-CoV-2**	TEXAS RED ¥	NFQ-MGB or None*	**20 µl** = 15 µl Master Mix + 5 µl extracted nucleic acids or controls†
N501Y mutation of *S gene*	VIC	NFQ-MGB or None*	
SΔ69-70 of *S gene*	FAM	NFQ-MGB or None*	
Exogenous IPC	Cy5	NFQ-MGB or None*	
To assign to samples and controls†			

* Depends on the thermal cycler model. Do not hesitate to contact the BioSellal Technical Support (tech@biosellal.com)
† Controls are NC (water), NCS (extracted water), EPC and or extracted MRI.
¥ Depending on the thermal cycler model, choose the ROX channel.

Table 6. UK & N501Y Variants Amplification program		
Standard ramping		
Cycles	**Time**	**Temperature**
1 cycle	20 min	50°C
1 cycle	5 min	95°C
40 cycles	10 sec	95°C
	45 sec + data acquisition	**63°C**

RESULTS INTERPRETATION

To analyze and interpret the signals obtained by qRT-PCR, the Threshold must be set up.
The threshold must be assigned carefully in order to obtain the most reproducible result between different manipulations. A consistent set of positives controls, usually an In-house Reference Material (MRI) or the EPC, is used to set the threshold value above the baseline and in the exponential amplification phase of the plot.
The Threshold Cycle, named « Ct » or « Cq » (depending on thermal cyclers), corresponds to the intersection between the amplification curves and the threshold line. It allows the relative measurement of the concentration of the target in the PCR reaction when a calibrated extract is analyzed in the same series.
The qRT-PCR series is validated if the controls (EPC, MRI, NCS and NC) present valid results, then the result of each sample can be interpreted.

Main Scenarios

Controls Reading

	Table 7. PCR Controls results interpretation				
	Targets				
	E gene TEXAS RED)	N501Y mutation (VIC)	SΔ69-70 deletion (FAM)	Exogenous IPC (Cy5)	Interpretation
NCS Negative Control Sample **MANDATORY**	Neg	Neg	Neg	Pos¥	Valid
	At least one of the three targets **Pos**			Pos¥	Contamination with a positive sample during extraction step or during qPCR plate preparation. In this case, it is recommended to repeat the samples extraction and add NCS in larger numbers, then repeat the RT-PCR assay. If the contamination persists, it may come either from environmental contamination of your lab or from the Master Mix. The latter possibility can be assessed by depositing 24 to 48 NC without any samples or control. If this test is positive, discard the tube of Master Mix
	Neg	Neg	Neg	Neg	Omission of exogenous IPC addition? Defective extraction ?
NC Negative PCR Control **OPTIONAL**	Neg	Neg	Neg	Neg	Valid
	At least one of the four targets **Pos**				Contamination with a negative or a positive sample during PCR plate preparation? or Master Mix / Water contamination? To test this hypothesis, it is recommended to deposit 24 to 48 NC without any samples or control. If this test is positive, discard the tube of Master Mix.
EPC PCR external positive control **MANDATORY** *IN ABSENCE OF MRI*	Pos*	Pos*	Pos*	Neg	Valid
	Neg	Neg	Neg	Neg	Problem during qRT-PCR plate preparation: Master Mix error? EPC omission?
	Pos*	Pos*	Pos*	Pos	Contamination with a sample during qRT-PCR plate preparation?
Sample process positive Control MRI	Pos†	Pos†	Pos†	Pos¥	Valid
	Neg	Neg	Neg	Neg	Problem during qRT-PCR plate preparation: Master Mix error? Nucleic acids extract omission or extract not in contact with Master Mix? Process drift: extraction and/or qRT-PCR ?
RECOMMENDED *IF AVAILABLE*	Neg	Neg	Neg	Pos¥	Process drift: extraction (in case of exogenous IPC addition directly into qRT-PCR plate and not during extraction) Problem withMRI preparation? Degradation of the sample process positive control?

* The Ct value obtained must be conform with the value indicated on the Certificate of Analysis (CA).
† The Ct value must be included within control card limits.
¥ The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. IPC Ct values for recommended extraction protocols are available upon request. BioSellal recommends you determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

Samples Reading

Table 8. Different types of results obtained for the samples				
Targets				
E gene (TEXAS RED)	N501Y mutation (VIC)	SΔ69-70 deletion (FAM)	Exogenous IPC (Cy5)	Interpretation
Neg	Neg	Neg	Pos*	**Negative or Undetected** Wrong sample chosen? Sample degradation?
Pos	Neg	Neg		**Detection** Of a strain other than 20I/501Y.V1 (UK), 20H/501Y.V2 (South-African) et 20J/501Y.V3 (Brazilian) variants
Pos	Pos	Neg		**Detection** Of a strain with suggestive mutations of 20H/501Y.V2 (South-African) et 20J/501Y.V3 (Brazilian) variants
Pos	Pos	Pos		**Detection** Of 20I/501Y.V1 (UK) variant
Pos	Neg	Pos		**Detection** Of another variant with SΔ69-70 deletion (e.g.: Denmark variant found in mink)
Pos	Pos	Pos	Neg or Ct>35	**Detection** Of 20I/501Y.V1 (UK) variant No quantification allowed Presence of inhibitors‡? Competition with the main target? Nucleic acids degradation in the sample?
Pos or Neg		Pos		**Detection** of SARS-CoV-2 genome with SΔ69-70 deletion. Evocative result of 20I/501Y.V1 (UK) variant or Denmark variant found in mink. IPC exogenous omission during the extraction and/or qRT-PCR Presence of inhibitors‡? Competition with the main target?
Pos or Neg		Neg		**Uninterpretable = Repeat the analysis** Nucleic acids extract omission or extract not in contact with Master Mix? Presence of inhibitors‡? Nucleic acids degradation in the sample? Problem during the IPC addition?

* The obtained Ct value depends on the thermal cycler, the sample type and the used extraction protocol. This value must be, at least, included within the specified range in the certificate of analysis (CA). IPC Ct values for recommended extraction protocols are available upon request. BioSellal recommends you determine your own maximal IPC Ct value depending on your own extraction method and thermal cycler.

‡ In case of inhibition suspicion, 1) Repeat the qRT-PCR with the dilution of extracted nucleic acids at 1/10 or 1/100 in the DNase/RNase free water. 2)Restart the analysis from the extraction step.

BIO-T KIT® SARS-CoV-2 UK & N501Y Variants PERFORMANCES

Diagnostic Specificity and Sensitivity on known status samples

The diagnostic specificity and sensitivity of the Bio-T Kit® SARS-CoV-2 UK & N501Y Variants was determined:

- *In sillico* by analysing all sequences available in public databases (GisAID,GenBank, NCBI) and performing alignments using MegAlign Pro software (DNAstar® Lasergene®). The *in-silico* specificity was confirmed by alignment using the online tool BLAST.
- *In vitro* from 82 samples found positive for SARS-CoV-2 using the Bio-T Kit® TriStar Covid-19. The variants status of the sample was sent by two partner laboratories using a reference method. Among these samples, 4 were found 20H/501Y.V2 (South-African) or 20J/501Y.V3 (Brazilian), 33 were found 20I/501Y.V1 (UK) and 45 were found SRAS-CoV-2 positive for other strain than 20I/501Y.V1, 20H/501Y.V2, 20J/501Y.V3. These sample were analysed with Bio-T Kit® SARS-CoV-2 UK & N501Y Variants on AriaMx™ (Agilent Technologies), ABI PRISM® 7500 Fast (Applied Biosystems) and QuantStudio 5 Real Time PCR system (Applied Biosystems) devices.

The results are analysed and expressed:

- For diagnostic specificity (Sp): in percentage of negatives found among the expected negatives.
- For diagnostic sensitivity (Se): in percentage of positives found among the expected positives.

		Known Status		
		Virus other than* 20I/501Y.V1 20H/501Y.V2, 20J/501Y.V3 *variants	**Suggestive mutation of 20H/501Y.V2 or 20J/501Y.V3 variants**	**20I/501Y.V1 variant**
Results with Bio-T kit® SARS-CoV-2 UK & N501Y Variants	**Virus other than 20I/501Y.V1 20H/501Y.V2, 20J/501Y.V3 variants**	**45**	**0**	**0**
	Suggestive mutation of 20H/501Y.V2 or 20J/501Y.V3 variants	**0**	**4**	**0**
	20I/501Y.V1 variant	**0**	**0**	**33**
	Total	**45**	**4**	**33**

⇨ **The Bio-T Kit® SARS-CoV-2 UK & N501Y Variants Diagnostic Specificity (Sp) is 100 %.**

⇨ **The Bio-T Kit® SARS-CoV-2 UK & N501Y Variants Diagnostic sensitivity (Se) is 100 %.**

Notes :

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