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Produktinformation

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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Clonit'*nGo* Dengue Virus

Detection of the genome of Dengue Virus using *Real Time PCR*

IVD	*In vitro* diagnostic device
	Revision 1 –25th May 2020
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	CLNG-96-70
	CLONIT srl Via Lombardia 6 – 27010 Siziano (PV) Via Varese 20 – 20121 Milano (MI)
Σ	96Tests

INTRODUCTION AND PURPOSE OF USE

Clonit'ngo Dengue Virus is designed for specific identification of Dengue virus in clinical samples from patients with signs and symptoms of Dengue virus infection. This test is intended for use as an aid in the diagnosis of the Dengue virus in combination with clinical and epidemiological risk factors. RNA is extracted from specimens, amplified using RT-PCR and detected using fluorescent reporter dye probes specific for Dengue virus.

CONTENT

The kit contains reagents enough to perform 96 amplification tests

	Quantity	Description
R1	12 x 8 well strips	Dengue Virus 8-well strips
R2	1 vial	Dengue Virus – positive control
R3	1 vial x 1.8ml	Rehydratation Buffer (Buffer B)
R4	1 vial x 1ml	Negative control (Buffer C)
R5	1 vial x 1ml	Water RNAse/DNAse free (Buffer A)
	12 x 8	8-cap strips

Instructions for Use: ST. CLNG-9670.ENG.1

MATERIALS AND STRUMENTATION REQUIRED BUT NOT SUPPLIED

Disposable latex powder-free gloves or similar material;
Bench microcentrifuge (12,000 - 14,000 rpm);
Micropipettes and Sterile tips with aerosol filter;
Vortex;
Plastic materials (microplate and optical adhesive cover);
Heat block (only for extraction)

INSTRUMENT

Clonit'nGo Dengue Virus is compatible with the following real time PCR instruments:

AriaMx/Aria Dx Real-Time PCR System fornito da Agilent Technologies
7500 Fast supplied from Lifetechnologies (1)
7500Dx Fast supplied from Lifetechnologies (1)
QuantStudio™12 Flex supplied from Lifetechnologies
QuantStudio™6 Flex supplied from Lifetechnologies
QuantStudio™7 Flex supplied from Lifetechnologies
QuantStudio™ 3 Fast supplied from Lifetechnologies
QuantStudio™ 5 Fast supplied from Lifetechnologies
StepOne Plus™ /StepOne™ supplied from Lifetechnologies (4)
ViiA™ 7 Fast supplied from Lifetechnologies

Exicycler™ 96 supplied from Lifetechnologies
CFX 96™ Real Time PCR supplied from BioRad
Mini Opticon™ supplied from BioRad
SmartCycler® supplied from Cepheid (2)
Rotor-Gene Q MDx® supplied from Qiagen (2)
LightCycler ®480 Real-Time PCR System supplied from Roche (3)
LightCycler ®96 Real-Time PCR System supplied from Roche (3)
Cobas z480 Analyzer supplied from Roche (3)

(1)Select Ramp Speed "**Standard**".
(2)The product should be reconstituted following the appropriate procedure (see Test Procedure) and transferred into the specific Rotor-Gene® Q or SmartCycler® tubes.
(3)Shell Frame grid plate which fits in these Roche qPCR System is necessary.
(4)No detection in Cy5 channel.

Please ensure that the instruments have been installed, calibrated, checked and maintained according to the manufacturer's instruction and recommendations

SAMPLE AND STORAGE

The product **Clonit'nGo Dengue Virus** is designed to be used with RNA extracted from biological samples. Perform sample preparation according to the recommendations in the "instructions for use" of the extraction kit used.

PREACAUTION USE

This kit is for in v*itro* diagnostic (IVD), for professional use only and not for in vivo use.
At all times follow Good Laboratory Practice (GLP) guidelines.
Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.
Avoid any contact between hands and eyes or nose during specimens collection and testing.
Handle and dispose all used materials into appropriate bio-hazard waste containers. It should be discarded according to local law.
Keep separated the extraction and the reagents preparation.
Never pipette solutions by mouth.
Avoid the air bubbles during the master mix dispensing. Eliminate them before starting amplification.
Wash hands carefully after handling samples and reagents.
Do not mix reagents from different lots.
It is not infectious and hazardous for the health (see Material Safety data Sheet – MSDS).
Do not eat, drink or smoke in the area where specimens and kit reagents are handled.
Read carefully the instructions notice before using this test.
Do not use beyond the expiration date which appears on the package label.
Do not use a test from a damaged protective wrapper.

LIMIT OF THE METHOD

The extreme sensitivity of gene amplification may cause false positives due to cross-contamination between samples and controls. Therefore, you should:

- physically separate all the products and reagents used for amplification reactions from those used for other reactions, as well as from post-amplification products;
- use tips with filters to prevent cross-contamination between samples;
- use disposable gloves and change them frequently;
- carefully open test tubes to prevent aerosol formation;
- close every test tube before opening another one.

The proper functioning of the amplification mix depends on the correct collection, correct transportation, correct storage and correct preparation of a biological sample.

As with any diagnostic device, the results obtained with this product must be interpreted taking in consideration all the clinical data and other laboratory tests done on the patient.

A negative result obtained with this product suggests that the RNA was not detected in nuclei acid extracted from the sample, but it may also contain RNA at a lower title than the detection limit for the product (detection limit for the product, see paragraph on Performance Characteristics); in this case the result would be a false negative.

As with any diagnostic device, with this product there is a residual risk of obtaining invalid, false positives or false negatives results

STORAGE AND STABILITY

The kits can be shipped and stored from 2 to 40 °C until the expiration date which is stated on the label. Once the positive control has been re-suspended, store it at -20 °C. We recommend to separate it in aliquots to minimize freeze and thaw cycles. Keep components away from sunlight.

ANALYTICAL PROCEDURE

RNA EXTRACTION

Perform the sample preparation according to the recommendations appearing in the instructions for use of the extraction kit used.
For RNA extraction from blood samples you can use your manually or automatic routine optimized system. Also, you can use any commercially available RNA extraction kit and follow the manufacturer´s instructions for use. We have validated the following extraction kits:

- Maxwell® 16 Viral Total Nucleic Acid Purification Kit, using the Maxwell® 16 instrument (Promega).
- QIAamp Viral RNA Mini Kit (Qiagen).
- ZP02003 MagPurix Viral Nucleic Acid Extraction Kit and ZP02013 MagPurix Viral RNA Extraction Kit.

POSITIVE CONTROL

Clonit'nGo Dengue Virus Positive Control contains high copies of the template, the recommendation is to open and manipulate it in a separate laboratory area away from the other components. Reconstitute the lyophilized Positive Control (red vial) by adding 100 µL of the supplied Water RNAse/DNAse free (white vial) and vortex thoroughly.

Once the positive control has been re-suspended, store it at -20°C. We recommend to separate it in aliquots to minimize freeze and thaw cycles.

PRECEDURE

Determine and separate the number of required reactions including samples and controls.
One positive and negative control must be included in each run for each assay.
Peel off protective aluminium seal from plates or strips.
Reconstitute the number of wells you need. Add 15 µL of Rehydration Buffer (blue vial) into each well.
Adding 5 µl of RNA extracted for each sample, 5 µl of positive control and 5 µl of negative control in different wells and close them with provided caps.
It is recommended to briefly centrifuge
Load the strips in the thermocycler.

Set up the thermocycler. Program the thermocycler following the conditions listed below and start the run:

SOFTWARE SETTINGS

Set the right thermal cycling:

Cycles	***Retrotrascription***			
1	*45°C 15 min*			
Cycles	***Denaturation***		***Annealing/ extension***	
1	*95°C 2 min*		*Reading stage*	
45	*95°C*	*10 sec*	*60°C*	*50 sec*

Fluorogenic data should be collected during the extension step following the instruction in the table:

	Denaque Virus	***Internal Control***
7500 Lifetech.	*FAM*	*VIC*
CFX 96	*FAM*	*HEX/JOE*
RotorGene Q	*Green*	*Yellow*
QS5 *Lifetech*.	*FAM*	*VIC*
Roche LightCycler	*465/510*	*533/580*

Depending on the equipment used select the proper detection channel. In Applied Biosystems 7500 Fast Real-Time PCR System, StepOne Plus™ Real Time PCR System and Stratagene Mx3005P ™ Real Time PCR System check check for information purposes only that passive reference option ROX is none. In the Applied Biosystems 7500 Fast Real-Time PCR System select Ramp Speed Standard in Select New Experiment/Advanced Setup/Experiment Properties.

Reaction Volume: 20 µl

RESULTS INTERPRETATION

The use of positive and negative controls in each run, validate the reaction by checking the absence of signal in the negative control well and the presence of signal for Dengue Virus in the positive control well. Check Internal Control signal to verify the correct functioning of the amplification mix. The analysis of the samples is done by the
software of the used real time PCR equipment itself according to manufacturer´s instructions.

Interpretation of results:

Dengue Virus (FAM)	***Internal Control (HEX)***	***Negative Control***	***Positive Control***	***Interpretation***
+	*+/-*	-	*+*	Dengue Virus Positive
-	*+*	-	*+*	Dengue Virus Negative
-	-	-	*+*	Fail
+	*+*	*+*	-	Fail

PERFORMACES

Clinical sensitivity and specificity

The clinical performance of **Clonit'nGo Dengue Virus** Kit was evaluated using different EQA panels (QCMD and INSTAND panels). The clinical performance of **Clonit'nGo Dengue Virus** was tested using 40 clinical specimens. The results were compared with the final EQA Reports:

		EQA reports		
Clonit'nGo Dengue Virus		*+*	*-*	*Total*
	+	*28*	*0*	*28*
	-	*0*	*12*	*12*
	Total	*28*	*12*	*40*

In conclusion, the results show a high sensitivity and specificity to detect Dengue Virus using **Clonit'nGo Dengue Virus.**

Analytical sensitivity:

For the purposes of this evaluation, the greater dilution (title) to which a positive sample can be diluted without the system losing its ability to detect it as positive is considered analytical sensitivity. **Clonit'nGo Dengue Virus** has a detection limit> 10 copies of RNA per reaction.

Analytical specificity:

Test's specificity is guaranteed by the use of specific primers for the target
The alignment of the choose regions for specific primers' hybridization with available sequences of present in database, demonstrated: their conservation and the complete specificity for the analyzed targets.

Crossreactivity

An analysis was also performed on samples positive for other pathogens and the test was performed following the indications reported in the method.

Samples	Results	Samples	Results
Zika Virus strain 11474/16 (French Polynesia)	-	St Louis Encephalitis virus	-
Zika Virus strain 11468/16(French Polynesia)	-	West Nile virus strain NY99	-
Zika Virus (African)	-	West Nile virus Heja	-
Zika virus strain PF13/251013-18 (Asian)	-	West Nile virus Ug37	-

Samples	Results	Samples	Results
Chikungunya virus S27 Petersfield	-	Yellow Fever virus strain 17D	-
Plasmodium falciparum 3D7	-	Trypanosoma cruzi	-
Japanese *encephalitis*	-		-

INTERFERENCES

Verify that in the RNA extracted from the sample there is no contamination from mucoproteins and haemoglobin, to exclude possible inhibition of PCR reaction. The interference due to contaminants can be detected through a spectrophotometric analysis, verifying the ratio between the absorbance readings at 260 nm (maximum absorbtion of Nucleic Acids) and 280 nm (maximum absorbtion of Proteins). A pure RNA should have a ratio of approximately 2.

QUALITY CONTROL

It is recommended to include in each analytical run, as quality control of every extraction, amplification and detection step, an already tested negative and positive sample, or a reference material with known concentration

In accordance with the Clonit srl ISO EN 13485 Certified quality Management System, each lot of **Clonit'nGo Dengue Virus** is tested against predetermined specification to ensure consistent product quality.

TECHNICAL ASSISTANCE

For any question and support please contact our Technical support:

e-mail: info@clonit.it
phone: +39 02 56814413

IVD	*In vitro* diagnostic device
	Read the instruction's manual
	Range of temperature
	Use within (dd/mm/yyyy: year-month)
LOT	Lot (xxxx)
REF	Code
	Manufacturer
	Contains sufficient for <n> tests

Clonit'nGo Dengue Virus is CE marked diagnostic kit according to the European *in vitro* diagnostic directive 98/79/CE

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CE IVD

for *in vitro* diagnostic use