Multiple RT-PCR kit for detection of 2019-nCoV

Manufactured by:

NaGene Diagnosis 纳捷诊断

1. Company Introduction

HYCOR Biomedical and NaGene Diagnosis

Garden Grove, CA (United States)

Kassel (Germany)

Beijing (China)

HYCOR Biomedical

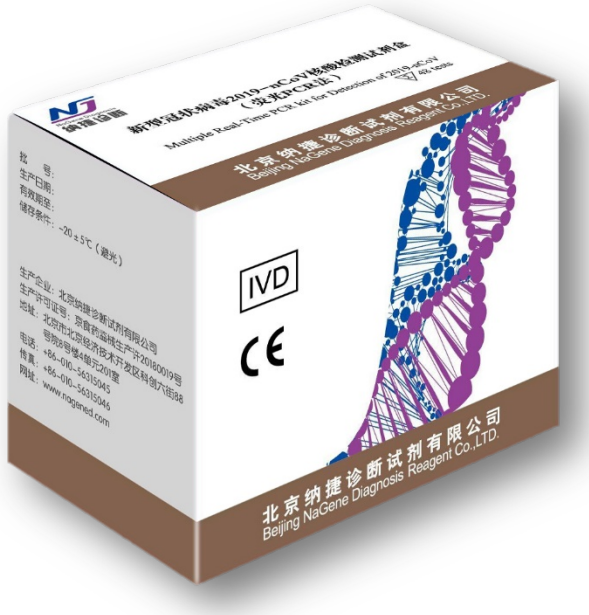
Founded in 1981, HYCOR is a global manufacturer and marketer of in vitro diagnostics products. Among its products, HYCOR markets the NOVEOS Immunoassay Analyzer and testing reagents for allergen sensitization testing, the first break though in routine allergy testing in 20 years, requiring 1/10 the sample per test compared to older methods. HYCOR also markets its HYTEC® legacy line of products by which millions of tests have been processed for clinicians around the world assessing patients for allergy or autoimmune diseases. Beyond the allergy focus, the company's response to the pandemic is to provide laboratories and healthcare systems with high quality COVID-19 testing, offering the highest value to clinicians and laboratories through innovation, reliability and customer service. For more information, please visit www.HYCORbiomedical.com

Beijing NaGene Diagnosis Reagent Co., Ltd

Beijing NaGene Diagnosis Reagent Co., Ltd was established in 2015 and is located in the Biomedical Park of Beijing Economic and Technological Development Zone. It is mainly engaged in the research, development, production and sales of molecular diagnostic reagent products. With the goal of "creating fast technology; setting up high-quality consciousness and making brand reagents", the company have cooperated with many well-known national research institutions and medical units to produce a new set of pathogenic nucleic acid extraction and fluorescent PCR amplification technology. The company is committed to providing fast, efficient, practical, complete, reliable, and innovative products and solutions for the clinical nucleic acid detection market. For more information, please visit www.nagened.com

2. Testing principles

Multiple RT-PCR kit for detection of 2019-nCoV



Intended Use:

The assay is used for the in vitro diagnosis for the detection of the ORF1ab and N genes of pneumonia suspected cases of novel coronavirus 2019-nCoV infection causing COVID-19 illness.

With Multiple Real-Time PCR kit, the samples are processed with compounded nucleic acid lysis buffer, which integrates nucleic acid lysis, RNase inhibition and RNA protection function, all together to achieve the “one-step” RNA detection of 2019-nCoV. In addition, RNA reverse transcription reaction and polymerase chain reaction (PCR) combined with TaqMan technology is used to amplify the target genes with specific primers according to the nucleic acid sequence of the virus.

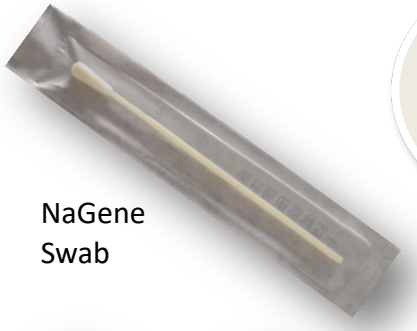
Time to result:

One of the advantages of this kit is its quick and easy processing of 96 samples with a time to result of 80 minutes.

Viral load sensitivity:
32 copies/mL

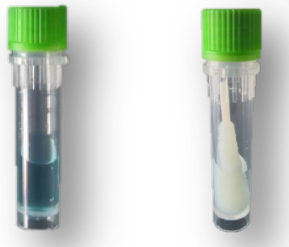
Validate PCR platforms: SLAN, ABI7500

3. Sample Requirements



NaGene Swab

1. Collect the samples with NaGene Swab



PB without swab

PB with swab

2. Put the swab into the NaGene Preservation Buffer

Box with ice

3 & 4. Store samples at 2-8 ºC & transport in sealed box with ice.

Sample Collection, Storage & Transport

1. Collect the samples with the swab:

IMPORTANT! Collection of both throat and nasopharynx samples: With **ONLY ONE SWAB** collect the sample, **first** wiping both tonsils and pharyngeal wall. **Then, with the same swab**, wipe the internal floor of the nose. Then put the swab in **NaGene's Preservation Buffer** for transportation to the laboratory. **ONLY one swab is needed** and ONLY one swab has to be introduced in NaGene Preservation Buffer tube.

How to collect the sample from the patient:

- **Throat** → Wipe both tonsils and posterior pharyngeal wall with swab at the same time, break off the swab head along the crease into the simple preservation solution tube. Discard the tail, and tighten the tube cover.
- **Nasopharynx** → Enter a flexible swab several centimeters with a slow, steady motion along the floor of the nose (straight back, not up the nose) until the posterior nasopharynx has been reached (distance from nostrils to external opening of ear). Place finger on the tip of the patient/resident's nose and depress slightly. Once resistance is met (the swab should pass into the pharynx relatively easily), rotate the swab several times and withdraw the swab
- **Sputum** → Dip the swab into the sputum, then break off the swab head along the crease into the sample preservation solution tube. Discard the tail, and tighten the tube lid.

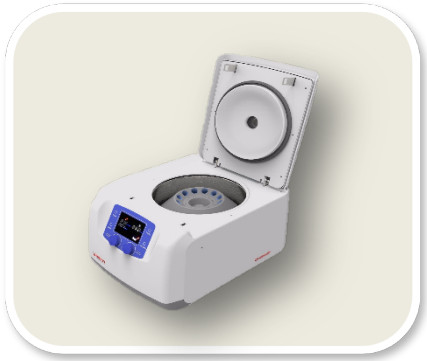
2. Insert the swab in NaGene's preservation solution tube

3. **Store samples** at 2-8 ºC for NO MORE than 24 hours. It it's more than 24 hours before testing, store at -20ºC. If it's more than 5 days before testing, store at -70/-80ºC.

4. **Transport** samples in a sealed box or container containing ice. The transportation shall comply with the relevant national biosafety regulations on class II pathogens.

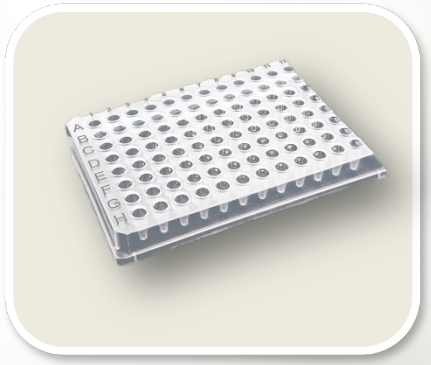
4. Supplies and accesories needed

Required but not provided with the kit:



Centrifuges

Pipettes
and tips



PCR Well
Plates

Thermocycler

Installation and electricity requirements:

The Multiple Real-Time PCR kit of NaGene does not use any connected instrument when it's being prepared. However, for the amplification, the use of a thermocycler is required. The electrical requirements for the thermocycler must be checked in the instrument technical specifications section of each individual thermocycler brand.

4. Supplies and accesories needed (cont.)

List of validated and non-validated platforms.

Validated

ABI7500	validated
SLAN-96P	validated
SLAN-96S	validated
SLAN-48P	validated

Non - Validated

Roche Lighcycler 480	need validation by customer
Rotor-GeneQ5plex	need validation by customer
Bio-Rad CFX96	need validation by customer
QuantStudio DX	need validation by customer
QuantStudio5	need validation by customer
Mx3005P/3000P	need validation by customer
Gentier 48E	need validation by customer
Gentier 48S	need validation by customer

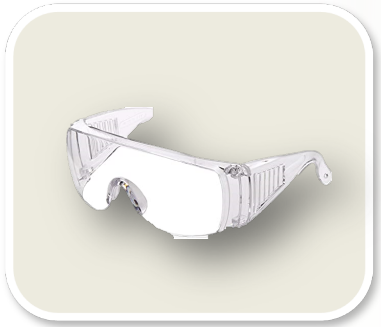
5. Biosafety Requirements

Personal Protection Equipment (PPE)

Masks

Gloves

Labcoat



Protective glasses/shield

Class II Biosafety Cabinet (BSC)

Source: Centers for Disease Control and Prevention, 2020. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). Coronavirus Disease 2019 (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

5. Biosafety Requirements (cont.)

Biosafety level required

The CDC recommends that clinical laboratories handling patient samples such as respiratory specimens, blood (and blood constituents), and urine, practice **Standard Precautions** within a BSL-2 facility. Additionally, work involving full-length genomic RNA should also be carried out at BSL-2

Appropriate disinfection requirements

Presently the CDC recommends utilizing disinfectants recognized on the Environmental Protection Agency's (EPA) List N, of which all are approved for use against SARS-CoV-2. Many List N disinfectants contain hydrogen peroxide, alcohol, bleach, or quaternary ammonium constituents. Consult with the disinfectant manufacturer if unsure about the suitability of a particular agent against SARS-CoV-2

Disposal requirements of the plates

The CDC and WHO recommends the handling of laboratory waste from testing suspected or confirmed COVID-19 patient specimens as all other biohazardous waste in the laboratory. Currently, there is no evidence to suggest that this laboratory waste needs any additional packaging or disinfection procedures

Source: Centers for Disease Control and Prevention, 2020. Interim Laboratory Biosafety Guidelines for Handling and Processing Specimens Associated with Coronavirus Disease 2019 (COVID-19). Coronavirus Disease 2019 (COVID-19). Available at: https://www.cdc.gov/coronavirus/2019-ncov/lab/lab-biosafety-guidelines.html

5. Testing kit Handling

1. Storage requirements

- Storage conditions: Reagent Component should be stored at - 20 ± 5 ℃ in dark.
- Preservation buffer stored between 2-30 ºC.
- Validity: tentatively 6 months.
- The production date and expiration date are shown in the outer package.
- The repeated freezing and thawing of the kit should not exceed 3 times.

2. Testing Kit appearance and number of test per box

Kit Box and components

Preservation Buffer

48 test / box

3. Description of kit components

Component name	Specification
Preservation Buffer	700 µl x 48
RT-PCR Reagent	1.70 mL x 1
Enzyme Mixture	100 µl x 1
Negative Control	110 µl x 1
Positive Control	110 µl x 1
Lysis Buffer (Internal Control inside)	300 µl x 1

4. Precautions on testing assay handling

If the specimen is collected with other type of commercial Virus Transport Media the extraction and purification of nucleic acids it is recommended.

7. Specimen collection

1. Collect the samples with the swab:

IMPORTANT! Collection of both throat and nasopharynx samples: With **ONLY ONE SWAB** collect the sample, **first** wiping both tonsils and pharyngeal wall. **Then, with the same swab**, wipe the internal floor of the nose. Then put the swab in **NaGene's Preservation Buffer** for transportation to the laboratory. **ONLY one swab is needed** and ONLY one swab has to be introduced in NaGene Preservation Buffer tube.

How to collect the sample from the patient:

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2. Insert the swab in NaGene's preservation solution tube

3. **Store samples** at 2-8 ºC for NO MORE than 24 hours. If it's more than 24 hours before testing, store at -20ºC. If it's more than 5 days before testing, store at -70/-80ºC.

4. **Transport** samples in a sealed box or container containing ice. The transportation shall comply with the relevant national biosafety regulations on class II pathogens.

8. Pre-Sampling techniques

The pre-sampling protocol that must be followed before starting is as follows:

1. PCR Reagent preparation (performed in Reagent Preparation Area)

- **1.1 Thaw RT-PCR Reagent and Enzyme mixture** in dark, at room temperature.
  - Vortex and centrifuge instantaneously.
- **1.2 PCR mix preparation:** Prepare PCR reaction mixture (master mix) according to the proportion of:
  - 33µL of RT-PCR Reagent and
  - 2µL of Enzyme mixture for each test.

2. Specimen preparation and PCR Setup (performed in Specimen Preparation Area)

- **2.1 Thaw the negative control, the positive control, the specimen** or extract to be tested at room temperature.
  - **Vortex the tubes, negative and positive controls. *Vortex and centrifuge samples at least 2 minutes at 13000 RPM**
- **2.2 Add 5µl Lysis buffer** at the bottom of each PCR tube, then **add 10µl** of either the negative control, the positive control, and or the specimen or extract, respectively in different PCR tubes, **gently mix it for 2-3 times with pipette**, then leave it at room temperature for 5 minutes.
- **2.3 Add 35µl of the prepared PCR mix into each well/tube**, cover it and mix it upside down, then centrifuge instantaneously, to be ready for PCR amplification.

9. Running of the assay

1. Computer commands / patient info system. The settings protocol for amplification phase in the thermocycler is the following:

RT-PCR Amplification

1) Place the PCR tubes into the thermocycler, and the cycle parameter setting is as below

2) The reaction volume is 50µL, and the selection of fluorescence channel is as below:

Step	Temperature	Incubation	Time Cycles
1	50ºC	15 min	x 1
2	94ºC	3 min	x 1
3	94ºC	10 sec	x 42 (collect signal at 58ºC)
	58ºC	35 sec	

Fluorescence channel	A (ORF1ab gene)	B (Internal Control)	C (N gene)
Fluorescence	FAM	HEX / VIC	CY5

9. Running of the assay (cont.)

2. Indicators of successful assay

Under the situation that all quality controls are normal, the expected results are:

Quality Control	Fluorescence channel	Normal result (Ct value)
Negative control	A (ORF1ab gene) + C (N gene)	No numerical value
	B (Internal control)	With S-type amplification curve, Ct < 39
Positive control	A (ORF1ab gene) + C (N gene)	Ct ≤ 39 with S-type amplification curve

9. Running of the assay (cont.)

2. Indicators of successful assay

According to the information of the detection results, the specimen's final result is:

Channel			Results judgement
A (FAM)	B (VIC)	C (CY5)	
Ct ≤ 39	Ct < 39	Ct ≤ 39	2019-nCoV Positive
No Ct or Ct>39	Ct < 39	No Ct or Ct > 39	2019-nCoV Negative
No Ct or Ct>39	Ct < 39	Ct ≤ 39	Retest
Ct ≤ 39	Ct < 39	No Ct or Ct > 39	

3. Use of the Software

It is recommended that the operator refers to the Operator's Manual when using the thermocycler software

9. Running of the assay (cont.)

4. Indication of plates error or rejection

Target genes	Internal Control	Result
−	+	The sample is **NEGATIVE**
−	−	**ABNORMAL RESULT: RETEST** Possible causes: existence of inhibitors in the PCR process.
+	+	The sample is **POSITIVE**
+	−	Normal results: **POSITIVE SAMPLE**. High titer positive samples will inhibit the amplification of IC, resulting in weak or negative results.

10. QC and QA

1. Internal Control

The Lysis Buffer contains the Internal Control. The interpretation is the following:

Quality Control	Fluorescence channel	Normal result (Ct value)
Negative control	A (ORF1ab gene) + C (N gene)	No numerical value
	B (Internal control)	With S-type amplification curve, Ct < 39
Positive control	A (ORF1ab gene) + C (N gene)	Ct ≤ 39 with S-type amplification curve

10. QC and QA (cont.)

2. Limit of detection (LoD).

The minimum detection limit of the kit is 32 copies / mL

Source: J de Jonge, B van der Veer, P van Kasteren, S van den Brink, L Wijsman, A van Esburg, J Murk, A Meijer (2020). Evaluation of NaGene COVID-2019 direct PCR kit for SARS-CoV-2 detection. National Institute for Public Health and the Environment (RIVM). Available from: http://www.nagened.com/newsitem/278501084

3. Targets of the assay

- ORF1ab gene (Fluorescence: FAM)
- N gene (Fluorescence: CY5)
- Internal Control gene (Fluorescence: VIC / HEX)

4. Need for EQA (External Quality Assessment).

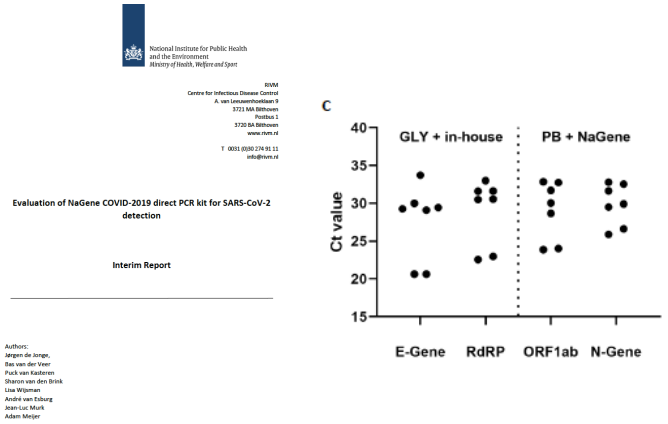
RIVM Evaluation of NaGene COVID-19 direct PCR kit for SARS-CoV-2 detection.

J de Jonge, B van der Veer, P van Kasteren, S van den Brink, L Wijsman, A van Esburg, J Murk, A Meijer (2020). Evaluation of NaGene COVID-2019 direct PCR kit for SARS-CoV-2 detection. National Institute for Public Health and the Environment (RIVM). Available from: http://www.nagened.com/newsitem/278501084

Machine/Platform preventive Maintenance

- HYCOR Biomedical is not responsible of the distribution of the thermocyclers.
- The thermocycler preventive maintenance must be carried out following the specifications of the Operator's Manual or by a Technical Service representative of the thermocycler company.

Nagene One Step PCR studies/trials/certificate


National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

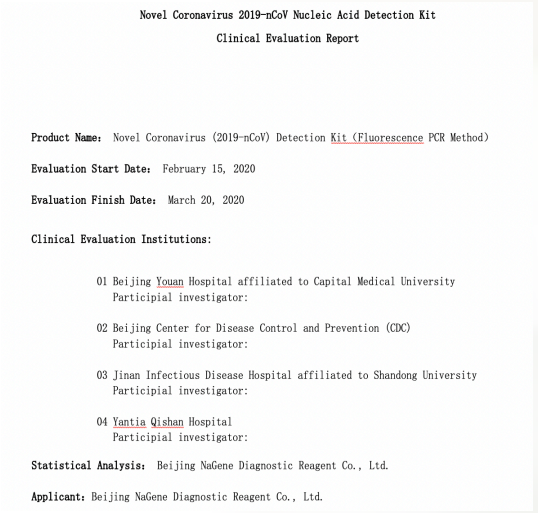
RIVM
Centre for Infectious Disease Control
A. van Leeuwenhoeklaan 9
3721 MA Bilthoven
Postbus 1
3720 BA Bilthoven
www.rivm.nl

T 0031 (0)30 274 91 11
info@rivm.nl

Evaluation of NaGene COVID-2019 direct PCR kit for SARS-CoV-2 detection

Interim Report

Authors:
Jørgen de Jonge,
Bas van der Veer
Puck van Kasteren
Sharon van den Brink
Lisa Wijsman
André van Esburg
Jean-Luc Murk
Adam Meijer


Novel Coronavirus 2019-nCoV Nucleic Acid Detection Kit

Clinical Evaluation Report

Product Name: Novel Coronavirus (2019-nCoV) Detection Kit (Fluorescence PCR Method)

Evaluation Start Date: February 15, 2020

Evaluation Finish Date: March 20, 2020

Clinical Evaluation Institutions:

01 Beijing Youan Hospital affiliated to Capital Medical University
Participial investigator:

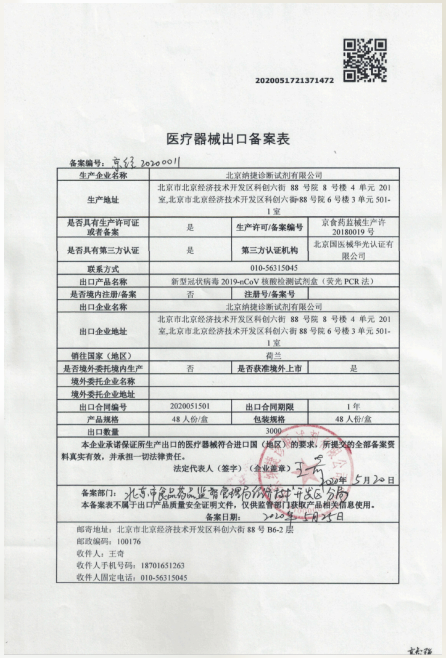
02 Beijing Center for Disease Control and Prevention (CDC)
Participial investigator:

03 Jinan Infectious Disease Hospital affiliated to Shandong University
Participial investigator:

04 Yantia Qishan Hospital
Participial investigator:

Statistical Analysis: Beijing NaGene Diagnostic Reagent Co., Ltd.

Applicant: Beijing NaGene Diagnostic Reagent Co., Ltd.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医疗器械出口备案表

备案编号：

生产企业名称	北京纳捷诊断试剂有限公司		
生产地址	北京市北京经济技术开发区科创六街 88 号院 8 号楼 4 单元 201 室,北京市北京经济技术开发区科创六街 88 号院 6 号楼 3 单元 501-1 室		
是否具有生产许可证或者备案	是	生产许可/备案编号	京食药监械生产许 20180019 号
是否具有第三方认证	是	第三方认证机构	北京国医械华光认证有限公司
联系方式	010-56315045		
出口产品名称	新型冠状病毒 2019-nCoV 核酸检测试剂盒（荧光 PCR 法）		
是否境内生产	否	注册号/备案号	
出口企业名称	北京纳捷诊断试剂有限公司		
出口企业地址	北京市北京经济技术开发区科创六街 88 号院 8 号楼 4 单元 201 室,北京市北京经济技术开发区科创六街 88 号院 6 号楼 3 单元 501-1 室		
销往国家（地区）	荷兰		
是否境外委托境内生产	否	是否获准境外上市	是
境外委托企业名称			
境外委托企业地址			
出口合同编号	2020051501	出口合同期限	1 年
产品规格	48 人份/盒	包装规格	48 人份/盒
出口数量	3000		

本企业承诺保证所生产出口的医疗器械符合进口国（地区）的要求，所提交的全部备案资料真实有效，并承担一切法律责任。

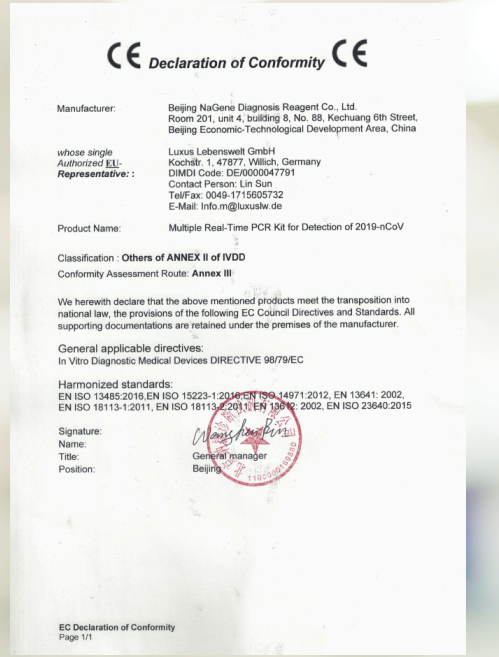
法定代表人（签字）（企业盖章） 2020 年 5 月 20 日

备案部门：

本备案表不属于出口产品质量安全证明文件，仅供监管部门获取产品相关信息使用。

备案日期：

邮寄地址：北京市北京经济技术开发区科创六街 88 号 B6-2 层
邮政编码：100176
收件人：王奇
收件人手机号码：18701651263
收件人固定电话：010-56315045


CE Declaration of Conformity CE

Manufacturer: Beijing NaGene Diagnosis Reagent Co., Ltd.
Room 201, unit 4, building 8, No. 88, Kechuang 6th Street, Beijing Economic-Technological Development Area, China

whose single Authorized EU-Representative: : Luxus Lebenswelt GmbH
Kochstr. 1, 47877, Willich, Germany
DIMDI Code: DE/0000047791
Contact Person: Lin Sun
Tel/Fax: 0049-1715605732
E-Mail: Info.m@luxuslw.de

Product Name: Multiple Real-Time PCR Kit for Detection of 2019-nCoV

Classification : **Others of ANNEX II of IVDD**
Conformity Assessment Route: **Annex III**

We herewith declare that the above mentioned products meet the transposition into national law, the provisions of the following EC Council Directives and Standards. All supporting documentations are retained under the premises of the manufacturer.

General applicable directives:
In Vitro Diagnostic Medical Devices DIRECTIVE 98/79/EC

Harmonized standards:
EN ISO 13485:2016,EN ISO 15223-1:2016, EN ISO 14971:2012, EN 13641: 2002, EN ISO 18113-1:2011, EN ISO 18113-2:2011, EN 1041: 2002, EN ISO 23640:2015

Signature:
Name:
Title: General manager
Position: Beijing

EC Declaration of Conformity
Page 1/1

National Institute of Public Heath and Environment Netherlands **(RIVM)** a WHO representative Laboratory

Sensitivity 91,2%
Specificity 100%
LoD is 32 copies / mL

589 Patient trial study
CDC Beijing, Capital Medical University, Shandong University Infectious Disease Hospital

Vs Clinical	Sensitivity	Specificity	Accuracy
Nasophargeal	90,48%	100%	96,56%
Sputum	93,75%	100%	96,30%

CFDA

CE IVD

Note : WHO EU application pending

THANK YOU

contact

info@promed.co.za