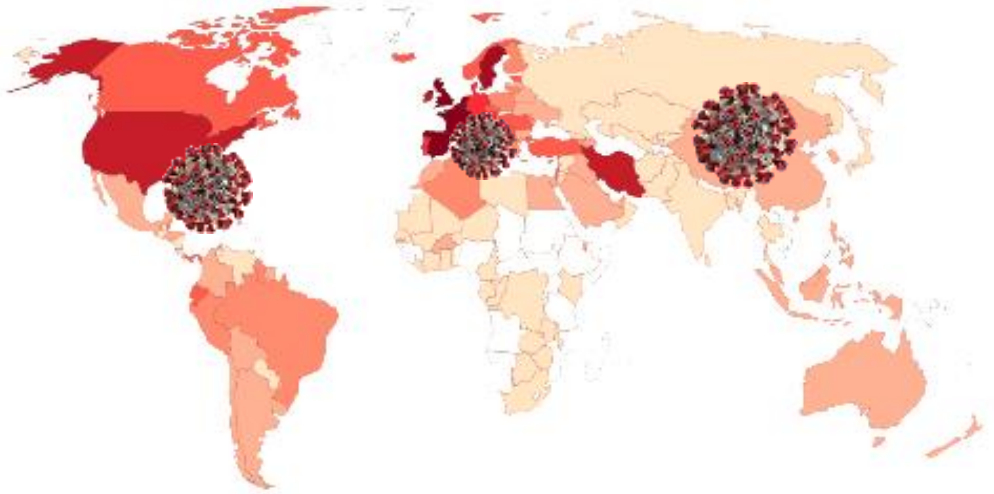
EuDx™ COVID-19 Detection Kit



CE IVD

Ministry of Food and Drug Safety

ISO 13485:2016

- Highly sensitive OneStep qRT-PCR Detection
- Result within 2 hours from extracted RNA
- Target SARS-CoV-2 specific (RdRp gene) RNA
- Does not detect other Coronavirus related viruses

Contents

Introduction

- Coronavirus Infection COVID-19 is a first-class infectious disease syndrome, defined as a respiratory syndrome caused by SARS-CoV-2 infection, and WHO declared a pandemic on March 11, 2020. Until now, it is known that it spreads through close contact with droplets or infected persons or can spread from contact with infected surfaces or objects. The incubation period is 1 to 14 days (average 5-6 days) and it is also contagious even symptoms dose not appear for 14 days after infection.

- There are no vaccines or specific antiviral agents yet, there for rely on conservative treatments such as fluid supplements and antipyretics. The mortality rate based on WHO is about 6.4%, and the elderly, patients with reduced immune function, and patients with underlying diseases are mainly lead to death.

- The pathogen is an RNA virus belonging to SARS-CoV-2: Coronaviridae and belongs to beta-CoV along with SARS and MERS. The genome of SARS-CoV-2 is a 29,891 nucleotide, +ssRNA encoding 9,860 amino acids, consisting of a 5'-capping structure and a 3'-poly-A tail at both ends and codes for four types of structural protein. The S protein binds specifically to the host cell receptor, the Nucleoprotein(N) protein binds to the RNA genome to make nucleocapsid, the membrane(M) protein connects between the membrane and capsid, and the envelope(E) protein involves in viral assembly, eruption, and constitutes the outer shell.

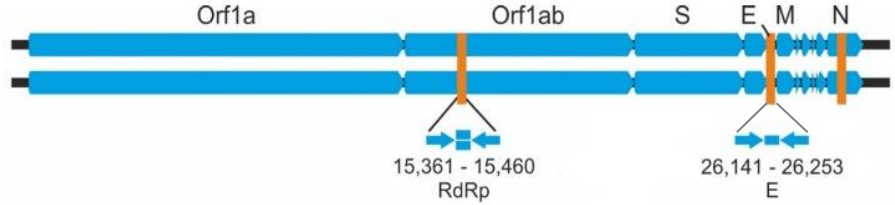
- SARS-CoV-2 showed 89.1% similarity to bat-derived SARS derived from bats and 79% similarity to SARS-Cov in comparison of base sequences. Therefore, it is important to accurately distinguish and diagnose similar sequences shared with the same species. Also, it is necessary to use an one-step RT qPCR reaction, considering the characteristics of POCT.

- Through using EuDx^{TM} COVID-19 Detection Kit , the virus can be effectively diagnosed by increasing the specificity and sensitivity with reducing the reaction time through comparative analysis of the gene sequences of SARS-CoV-2 and SARS, MERS and other RNA viruses. This product is intended to contribute to the diagnosis, treatment and eradication of COVID-19, which is pandemic worldwide.

I. Principle

EuDx™ COVID-19 Detection Kit is an in vitro diagnostic reagent for to qualitative detection of *RdRp* gene of COVID-19(SARS-CoV-2) and *E* gene of *Betacoronavirus* from extracted RNA from Nasopharyngeal swab, Oropharyngeal swab or Sputum by OneStep qRT-PCR.

■ Detection Target



Target Virus	Target Genes
SARS-CoV-2	*RdRp*
	E
	Internal Control

The kit includes 2x OneStep qRT-PCR Buffer, OneStep qRT-PCR Enzyme mix (including RTase, DNA polymerase and RNase inhibitor) and Primer & Probe Mixture. We also provide a Control Template (SARS-CoV-2) and Internal Control DNA(*Spinacia*), which allows us to compare the results with clinical sample results to ensure correct interpretation.

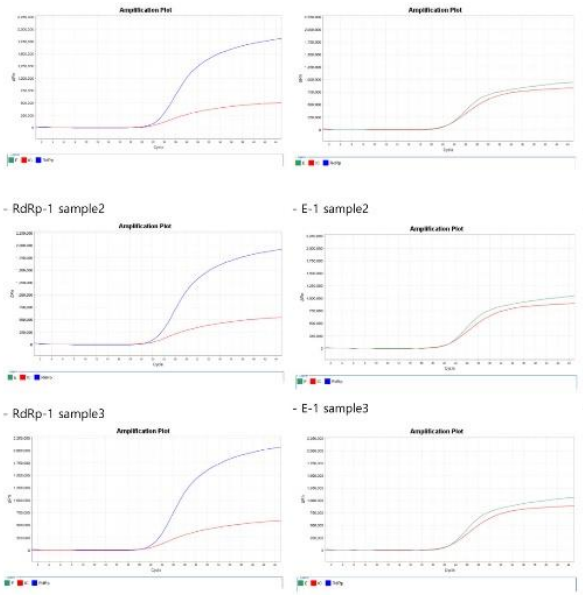
■ Fluorescence Information

Target Genes	5'Fluorephore	3'Quencher
RdRp	FAM	BHQ1
E	FAM	BHQ1
Internal Control	Cy5	BHQ2

■ PCR Platform

- ABI7500 (Thermo Fisher)
- Compatible instruments with FAM and Cy5 channels

II. EuDx™ COVID-19 Detection Kit



Model Name	Storage	Period of Use
EuDx™ COVID-19 Detection Kit	-20±5℃	12 months

EuDx™ COVID-19 Detection Kit should be stored at -20±5℃ and kept away from sunlight. Follow recommended storage conditions for all components.

- Each component of the product is valid for 12 months from the manufactured date.
- Do not use product beyond the expiration date.
- Please thaw the product on ice.
- Avoid multiple freeze-thaw cycle (over 5 times). After removing the amount you need, quickly freeze the rest right away to avoid degradation of the enzyme activity (e.g. quality of the product) caused by multiple freeze-thaw cycle.
- If there are or have been issues during transportation or the protective packaging is damaged, contact your dealer and follow their instructions.

■ Kit Contents

Components	Cap	Quantity
2x RT-PCR Master Mix		1㎖x2ea
4x Reaction Mixture-1	1	500㎕
4x Reaction Mixture-2	2	500㎕
Positive Control DNA		100㎕
Nuclease Free Water		1㎖

■ Material to be supplied by User

- Microcentrifuge
- Vortex mixer
- Pipettes/ pipette filter tips
- Centrifuge
- RNase free consumables: Disposable latex or vinyl gloves, sterile pipette tips
- Cooling device or ice
- Micro-centrifuge tube (1.5 ㎖)
- Tube or plates: Real-time PCR tube (0.1㎖ or 0.2 ㎖ depends on instrument type used)
- Viral RNA preparation kit

III. Protocol

■ Preparation and Treatment of Specimen

* All specimens are considered to be potent to transfer infectious agents.

- Specimens: Nasopharyngeal swab, Oropharyngeal swab, or Sputum.
- Collect specimens in an aseptic environment to prevent contamination.
- Keep the swab sterile to avoid changing the levels of RNA which should be proportional to the amount of the initial cells.
- To maintain stable condition, store the clinical specimen at an instructed temperature.
- Keep the collected specimens for 24 hours at 2~8 ℃ without any pre-treatment (Keep refrigerated if the pre-treatment cannot be done within 1 hour).

■ RNA Extraction

USE a commercially available RNA isolation kit (QIAGEN, etc.).
User should verify their own RNA extraction reagent

■ Preparation of EuDx™ COVID-19 Detection Kit

1) Please thaw all reagents on the ice. After vortex, spin down.
2) Prepare PCR Master Mix by adding the following reagent.
3) The amount of Master mix should be prepared by calculating additional amount corresponding to at least 1~2 reaction instead of the number including sample, control template (2019-nCoV), and NTC (Non-Template Control).
4) Mix PCR master mix using vortex and spin down.

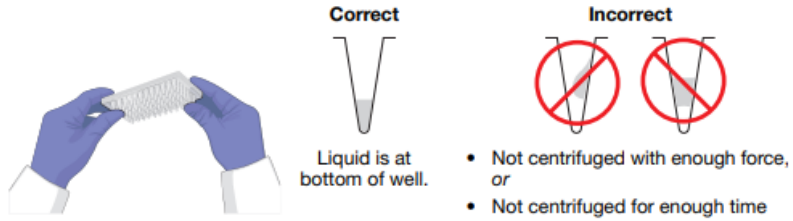
Component	1 rxn	5rxn	10rxn
2x RT-PCR Master Mix	10㎕	50㎕	100㎕
4x Reaction Mixture-1 or 2	5㎕	25㎕	50㎕
Total Volume	15㎕	75㎕	150㎕

Protect the Probe from the light. When the Probe is exposed to the light for a long time, fluorescence may be reduced and may affect the result.

5) Dispense 15 ㎕ into a plate or strip tube suitable for the equipment using the manufactured master mix. (Be careful of cross contamination)
6) Add 5㎕ of Clinical sample or Control template. Labeling should be done to avoid confusion of template position.

Component	Volume
PCR Master mix	15㎕
Sample	5㎕
Total Volume	20㎕

7) Close Close the PCR tube cap, and centrifuge concisely. Then make sure solution is collected at the bottom of the tube.



III. Protocol

■ Set-up and Running of the Device (ABI7500 Real-Time PCR Systems)

1) Open 7500 software and click **File → New Experiment** in Main Menu on the top of screen.
2) Write your file name in **blank** next to **Experiment Name** and then choose an experimental instrument.
3) Select **Plate Setup** and input the experimental conditions as follows.

① Selection of define Targets

Target	Dye	Quencher
RdRp(Reaction-1)	FAM	BHQ1
E(Reaction-2)	FAM	BHQ1
Internal Control(IC)	Cy5	BHQ2

② Setting of Assign Targets and Samples: select wells and click targets. Check that passive reference is none (at the bottom left of the screen).

③ PCR Run Condition enter 20㎕ for Sample Volume for Sample Volume.

Step	Number of cycles	Temperature(℃)	Duration
1	1	50	15 min
2	1	95	15 min
3	45	95	20 sec
4		58	40 sec

4) Open the **ABI7500** instrument, insert the **PCR tube** and close it.
5) Click '**Run**' in the Experiment Menu and click '**Start Run**' on the green button.

■ Result Analysis

1) ABI7500 Real-time PCR systems

① Threshold and Base line setting

Analyte	Fluorophore	Quencher	Threshold value	Base line
RdRp	FAM	BHQ1	10,000	3-20
E	FAM	BHQ1	15,000	3-20
Internal control (IC)	Cy5	BHQ2	5,000	3-15

② Data Analysis

Ct value	Result
<39	Detected (+)
≥ 39 or Undetermined	Not detected (-)

If the result is invalid, dilute the extracted RNA and run again. If it does not correct the test results, re-do the test from the start. Refer to "Troubleshooting".

Data analysis valid, when only the IC/QC results are valid

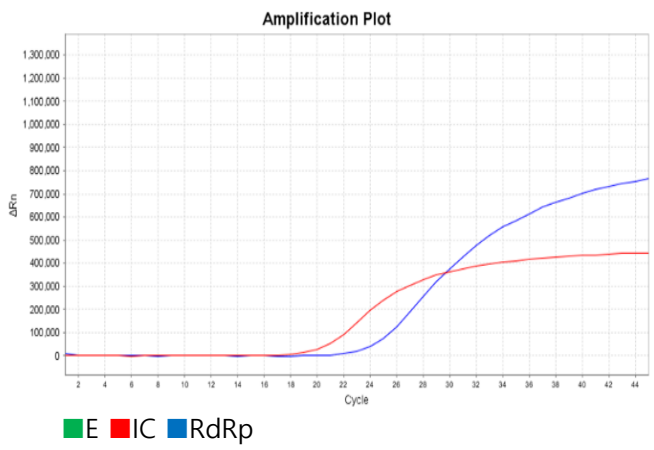
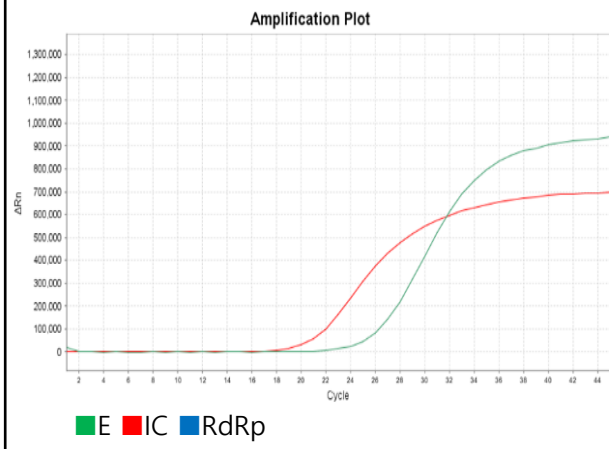
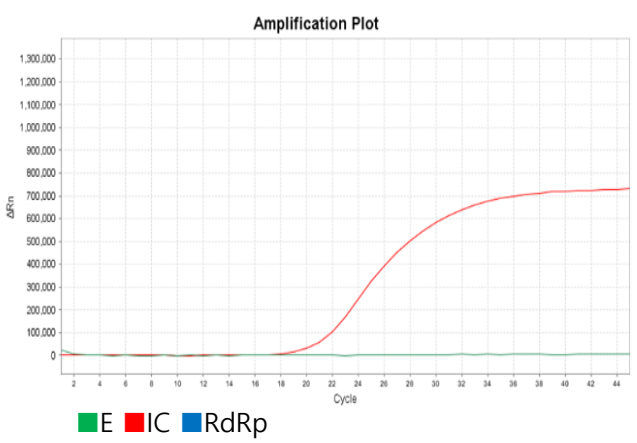
III. Protocol

Sample		Results (FAM)	IC (Cy5)	Detection	Comment
Specimen		Ct < 39 (Reaction 1 and 2)	Ct < 27	Positive	COVID-19 Positive
		Ct < 39 (Reaction 1)		Inconclusive	See below*
		Ct < 39 (Reaction 2)		Sarbecovirus Positive	See below**
		Ct≥39		Negative	Samples might be of low concentration or negative
		Ct < 39	Ct≥27	Re-test	Purify RNA samples again
Positive Control	Reaction 1	19.5 ± 3.0	Ct < 27	Normal	
	Reaction 2	19.5 ± 3.0			
Negative Control	All Reaction	Not detected			
	All Reaction	Detected	Ct < 27	Abnormal	Suspect the contamination

* If the repeated result is still the same, confirm the result using other methods such as viral sequencing.

** All Target Results are valid. Sarbecovirus RNA is detected but 2019-nCoV(SARS-CoV-2) specific RNA targets are not detected. Repeat testing. For samples with the same result on a repeated test, additional confirmatory testing may be conducted, if it is necessary to differentiate between 2019-nCoV (SARS-CoV-2) and SARS-CoV-1 or other Sarbecovirus currently unknown to infect humans, for epidemiological purposes or clinical management. Missing amplification of the 2019-nCoV (SARS-CoV-2) specific targets may be due to: 1) a sample at concentrations near or below the limit of detection of the test, 2) a mutation in the corresponding target region, or 3) other factors.

III. Protocol

Typical Results		
		
RdRp	E	IC

IV. Troubleshooting

Observation	Probable causes	Solution
Any other signals (including Internal control signal) are not observed	Nucleic acid extraction failure	Re-do the extraction
	Incorrect PCR cycle or machine temperature	Please check the PCR conditions and repeat the PCR under the correct setting if necessary
	Presence of inhibitor	Dilute the template nucleic acid in sterilized D.W. (10-100x) and repeat the PCR with diluted nucleic acid.
	Incorrect storage of the reagents	Check and verify the correct storage condition, and use new reagents.
nternal control signal is not observed	High load of pathogen’s nucleic acid	Dilute the template nucleic acid in sterilized D.W. (10-100x) and repeat the PCR with diluted nucleic acid.
	Presence of inhibitor	Dilute the template nucleic acid in sterilized D.W. (10-100x) and repeat the PCR with diluted nucleic acid (If specimen is still present, restart from nucleic acid extraction)
False positive or signals observed at the negative control	Presence of cross contamination	Decontaminate all surfaces and instruments with sodium hypochlorite and ethanol. Use only filter tips during extraction procedure. Change tips among tubes. Repeat the nucleic acid extraction with new set of reagents
False negative or no signals observed at the positive control	Defective Positive control	Avoid multiple freeze-thaw cycle

V. Warnings and Precautions

Safety Precautions in the Lab.

- ✓ Wear gown and gloves when handling infectious samples, and wash hands after work.
- ✓ Specimens used in the test are potentially infectious. It should be handled as if it were a contagious materials.
- ✓ To avoid contamination use disposable gloves and experiment tools (which could affect the diagnosis).
- ✓ Do not reuse disposables (e.g. tip, test gloves, test tubes, etc.) to avoid contamination.
- ✓ Avoid contact with this reagent to skin, eyes, or mucosa. In case of contact, flush with plenty of water.
- ✓ Wash hands after handling the specimens and reagents.
- ✓ Sterilize work table after the test.
- ✓ When opening reagent bottle or taking out contents, be cautious of cross contamination caused by microorganisms
- ✓ Follow storage method to store regents before and after use.
- ✓ Do not leave reagent bottles opened.
- ✓ Use the sterilized pipettes and tips.
- ✓ Dispose materials that could be contaminated according to the rules of safe disposal of contaminated materials.

Other Precautions

- ✓ Do not use the result obtained with this kit as a final diagnosis. The result should be used in parallel with other methods of diagnosis.
- ✓ PCR is highly sensitive and be handled with care to avoid cross contamination.
- ✓ Use change filter tips to avoid cross contamination when PCR and specimens are extracted. It is recommended to use separate pipettes.
- ✓ To prevent misuse of real time PCR device and abnormal result, inspect periodically to validate temperature sensor of the equipment. Always close cap when not in use.
- ✓ Use verified PCR devices only.

VI. Performance Evaluation

Limit of Detection (LOD)

The limit of detection was measured using plasmid DNA synthesized according to the sequence provided by NCBI. The following table shows the results of the tests conducted by testers A and B (2 people) 6 times (repeated twice).

RdRp gene				
Copies	10^4	10^3	10^2	10^1
Detect/total number of tests	24/24	24/24	23/24	11/24
Detectability (%)	100	100	96	46
E gene				
Copies	10^4	10^3	10^2	10^1
Detect/total number of tests	24/24	24/24	24/24	24/24
Detectability (%)	100	100	100	100

Clinical Evaluation

Samples Classification	Positives (Detected / Tests)	Negatives (Undetected /Tests)
Result	10 / 10	18 / 18

Clinical evaluation showed that '**EuDx™ COVID-19 Detection Kit**' has clinical validity for 28 COVID clinical samples (6 of 10 as 2019-nCoV positive, 4 of 10 as Sarbecovirus, and the remaining as negative.)

Cross Reactivity

The cross reactivity tests were performed using COVID-19 standard materials and negative reference materials. None of standard and negative materials were detected in any of the tests performed.

	Cross-Reactant		Cross-Reactant
1	*Influnenza A virus* (H1N1)	18	*Herpes simplex virus*
2	*Influnenza A v*irus (H1N1pdm09)	19	*Zika virus*
3	*Influnenza A virus* (H3N2)	20	*Chikungunya virus*
4	*Influnenza B virus* (Yamagata-like)	21	*Mycoplasma pneumoniae*
5	*Influnenza B virus* (Victoria-like)	22	*Legionella pneumophila*
6	*Influnenza B virus*,strain B/Lee/40	23	*Clamydophila pneumoniae* strain CM-1
7	*Influnenza B virus*,strain B/Taiwan/2/62	24	*Haemophilus influenzae*
8	*Human respiratory syncytial virus* stain 18537	25	*Streptococcus pneumoniae*
9	*Human respiratory syncytial virus* stain A2	26	*Mycobacterium intracellulare*
10	*Respiratory Syncytial virus*(HRSV-A)	27	*Mycobacterium tuberculosis*
11	*Respiratory Syncytial virus*(HRSV-B)	28	*Klebsiella pneumoniae*
12	*Human adenovirus* 1	29	*Pseudomonas aeruginosa*
13	*Human adenovirus* 2 strain Adenoid 6	30	*Haemophilus parainfluenzae*
14	Adenovirus type 5	31	*Neisseria meningitidis*
15	*Mumps virus*, strain Enders	32	*Staphylococcus epidermidis*
16	*Rhinovirus* A-2	33	*Bordetella pertussis*
17	*Enterovirus*	34	*Flu A* subtype(H5) pDNA

VII. Ordering Information

Products	no. of test	Cat. No.
EuDx™ COVID-19 Detection Kit	100 tests	COV-02-100T

Eudipia Co., Ltd.

#305 Research Center 2, 194-41 Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu

Cheongju-si, Chungcheongbuk-do, Korea

Tel : +82 70 8890 2101, Fax : +82 43 238 9448

E-mail : sales@eudipia.com

www.eudipia.com

DECLARATION OF CONFORMITY

MANUFACTURER : Eudipia Co., Ltd.
#306, Research Center 2, 194-41, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, Rep. of Korea

EUROPEAN REPRESENTATIVE : MT Promedt Consulting GmbH
Altenhofstrasse 80, 66386 St. Ingbert, Germany

PRODUCT : EuDx™ COVID-19 Detection Kit
CATALOG NO. : COV-02-100T

EDMA code/ Term : 15.04.40.90.00/Other Virology - NA Reagents

CLASSIFICATION : General IVD
(Neither Listed in Annex Ⅱ of IVDD, nor self-testing device)

CONFORMITY ASSESSMENT ROUTE : IVDD ANNEXⅢ (Self-Declaration)

We here with declare that the above mentioned products meet the provisions of the council directive 98/79/EC for In Vitro diagnostic medical device. All supporting documentation is retained under the premises of the manufacturer.

STANDARDS APPLIED : EN ISO 13612:2002, EN ISO 13641:2002, EN ISO 13485:2016, EN 13975:2003, EN 14971:2012, EN ISO 17511:2003, EN ISO 18113-1:2011, EN ISO 18113-2:2011, ISO 23640:2011

SIGNATURE : In-Soo Kim, CEO

Apr. 22. 2020.
Eudipia, Reblic of Korea

TÜVRheinland

Certificate

The Certification Body of
TÜV Rheinland LGA Products GmbH

hereby certifies that the organization

EuDiPia Co., Ltd.
#306, Research Center 2, 194-41,
Osongsaengmyeong 1-ro,
Osong-eup,Heungdeok-gu,
Cheongju-si,Chungcheongbuk-do 28160
Republic of Korea

has established and applies a quality management system for medical devices for the following scope:

Design and Development, Manufacture and Distribution of In-Vitro Diagnostic Reagents - Qualitative Molecular Diagnosis for Infectious Disease
(See attachment for site included)

Proof has been furnished that the requirements specified in

EN ISO 13485:2016

are fulfilled. The quality management system is subject to yearly surveillance.

Effective Date: 2019-03-28
Certificate Registration No.: SX 60137122 0001
An audit was performed. Report No.: 12031444 004
This Certificate is valid until: 2021-09-02

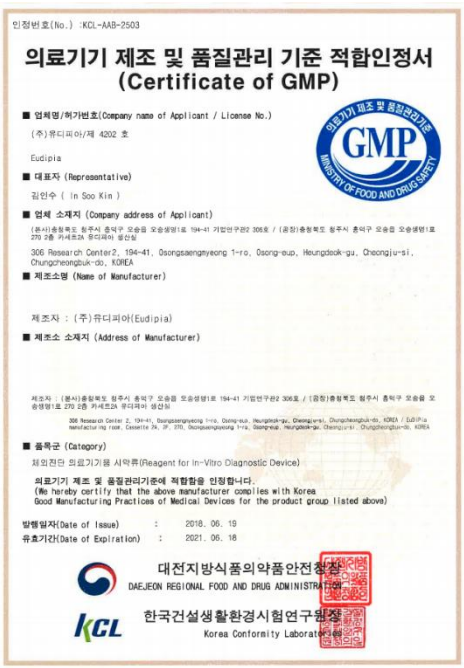
Certification Body

DAkkS Deutsche Akkreditierungsstelle D-ZM-14169-01-02

Masahiro Asami

Date 2019-03-28

TÜV Rheinland LGA Products GmbH - Tillystraße 2 - 90431 Nürnberg



인정번호(No.) :KCL-AAB-2503

의료기기 제조 및 품질관리 기준 적합인정서
(Certificate of GMP)

■ 업체명/허가번호(Company name of Applicant / License No.)
(주)유디피아/제 4202 호
Eudipia

■ 대표자 (Representative)
김인수 (In Soo Kin)

■ 업체 소재지 (Company address of Applicant)
(본사)충청북도 청주시 흥덕구 오송읍 오송생명1로 194-41 기업인구관2 306호 / (공장)충청북도 청주시 흥덕구 오송읍 오송생명1로 270 2층 카세트2A 유디피아 생산실
306 Research Center 2, 194-41, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, KOREA

■ 제조소명 (Name of Manufacturer)
제조자 : (주)유디피아(Eudipia)

■ 제조소 소재지 (Address of Manufacturer)
제조자 : (본사)충청북도 청주시 흥덕구 오송읍 오송생명1로 194-41 기업인구관2 306호 / (공장)충청북도 청주시 흥덕구 오송읍 오송생명1로 270 2층 카세트2A 유디피아 생산실
306 Research Center 2, 194-41, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, KOREA / EuDiPia manufacturing room, Cassette 2A, 2F, 270, Osongsaengmyeong 1-ro, Osong-eup, Heungdeok-gu, Cheongju-si, Chungcheongbuk-do, KOREA

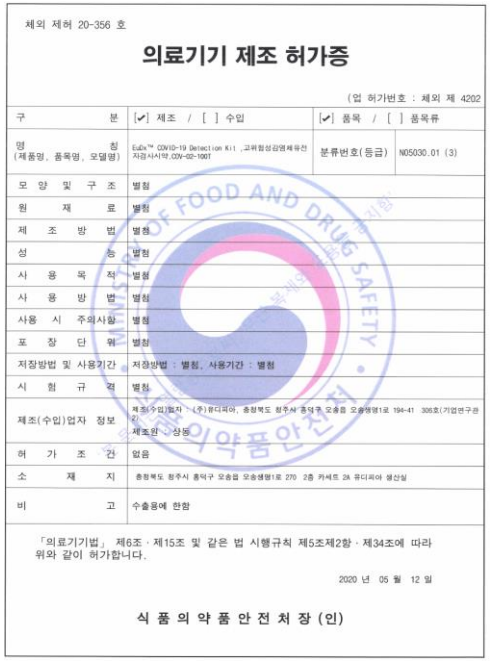
■ 품목군 (Category)
체외진단 의료기기용 시약류(Reagent for In-Vitro Diagnostic Device)

의료기기 제조 및 품질관리기준에 적합함을 인정합니다.
(We hereby certify that the above manufacturer complies with Korea Good Manufacturing Practices of Medical Devices for the product group listed above)

발행일자(Date of Issue) : 2018. 06. 19
유효기간(Date of Expiration) : 2021. 06. 18

대전지방식품의약품안전청장
DAEJEON REGIONAL FOOD AND DRUG ADMINISTRATION

KCL 한국건설생활환경시험연구원장
Korea Conformity Laboratory



체외 제허 20-356 호

의료기기 제조 허가증

(업 허가번호 : 체외 제 4202

구 분	[✔] 제조 / [ ] 수입	[✔] 품목 / [ ] 품목류	
명 칭 (제품명, 품목명, 모델명)	EuDx™ COVID-19 Detection Kit , 고위험성감염체유전자검사시약,COV-02-100T	분류번호(등급)	N05030.01 (3)
모 양 및 구 조	별첨		
원 재 료	별첨		
제 조 방 법	별첨		
성 능	별첨		
사 용 목 적	별첨		
사 용 방 법	별첨		
사용 시 주의사항	별첨		
포 장 단 위	별첨		
저장방법 및 사용기간	저장방법 : 별첨, 사용기간 : 별첨		
시 험 규 격	별첨		
제조(수입)업자 정보	제조(수입)업자 : (주)유디피아, 충청북도 청주시 흥덕구 오송읍 오송생명1로 194-41 306호(기업인구관 2) 제조원 : 상동		
허 가 조 건	없음		
소 재 지	충청북도 청주시 흥덕구 오송읍 오송생명1로 270 2층 카세트 2A 유디피아 생산실		
비 고	수출용에 한함		

「의료기기법」 제6조 · 제15조 및 같은 법 시행규칙 제5조제2항 · 제34조에 따라 위와 같이 허가합니다.

2020 년 05 월 12 일

식 품 의 약 품 안 전 처 장 (인)