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Wako

Code No. 283-33209 (200reactions)
Code No. 281-34501(200reactions)
Code No. 199-18991(50reactions)

For Research Use Only

SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)

SARS-CoV-2 Lysis Buffer Ver.2

Outline

Code No. 283-33209 (For 200 reactions, 100 samples)

SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)

SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene) is a reagent system, based on an one-step real-time PCR method, for the qualitative detection of SARS-CoV-2-derived RNA. The highly active enzyme applied in the kit allows high-speed real-time RT-PCR and it plays dual roles as reverse transcriptase and DNA polymerase.

The probe No.1 and 2 are designed to hybridize to SARS-CoV-2 N gene and labeled with the FAM. Each of the probe targets different sequences at N gene. Two wells are required for testing a sample.

【Storage】
－20℃

【Precautions】

- Be careful of safety according to a guideline at your laboratory.
- Wear safety glasses and gloves during the experiment.
- Handle all components and samples in a biosafety cabinet or a clean bench.
- Keep all components and samples on ice during the experiment
- Make certain that RNA is devoid of any RNase contamination and maintain aseptic conditions.
- Do not use buffer containing EDTA such as TE buffer.

【Materials required but not provided】

- Real-Time PCR instrument
- Microcentrifuge
- Micro pipette and nuclease-free pipette tips
- Nuclease-free sterilized water*

  *It is used for dilution of positive control RNA.
- Nuclease-free 1.5mL tubes*(e.g. Micro tube 1.5ml DNA LoBind, Eppendorf)

  *Low DNA/RNA binding tubes are recommended.
- Real-time PCR plates and plate films or real-time PCR tubes and caps.

【Components】

Components	Volume
5×Reaction Buffer	800μL
2mmol/L dNTPs	800μL
50mmol/L Manganese(II) Acetate	200μL
Hot Start Reverse Transcription DNA Polymerase	50μL
Distilled Water, Nuclease-free	870μL
Fw & Rv Primers 1	100μL
TaqMan® Probe 1	40μL
Fw & Rv Prmers 2	100μL
TaqMan® Probe 2	40μL
Positive Control RNA, N gene	400μL

TaqMan® is registered trademark by Roche Diagnostics K.K.

【RNA copy number of Positive Control RNA, N gene】

Indicated on the label.

*Copy number is quantificated by digital PCR using Naica™ System(Stilla Technologies).

【Fluorescence dyes】

Components	Reporter
TaqMan® Probe 1	FAM
TaqMan® Probe 2	FAM

【Protocol】

<Preparation of reagents>

1. Thaw the components in the kit to room temperature.
2. Mix each reagent by vortexing or pipetting to be homogeneous and spin down it.

<Preparation of samples>

Prepare purified RNA solution from the nucleic acid extraction.

Do not use buffer containing EDTA such as TE buffer for dilution for preparing purified RNA solution.

1. Positive control RNA

Prepare a working positive control RNA solution by diluting Positive Control RNA, N gene with nuclease-free water using nuclease-free 1.5mL tube.

Do not use buffer containing EDTA such as TE buffer for dilution.

2. PCR Master Mix

Prepare PCR master mix solution according to the following table.

The table indicates reagent volume when applying 5μL of RNA sample into a well(total 20μL/well). RNA sample volume can be increased with decreasing distilled Water, Nuclease-free volume(total 20μL/well).

PCR Master Mix No.1

Composition	For a well /μL	For 20 wells/μL
5×Reaction Buffer	4.00	80
2mmol/L dNTPs	4.00	80
50mmol/L Manganese(II) Acetate	1.00	20
Hot Start Reverse Transcription DNA Polymerase	0.25	5
Fw & Rv Primers 1	1.00	20
TaqMan® Probe 1	0.40	8
Distilled Water, Nuclease-free	4.35	87
Total	**15.00**	**300**

PCR Master Mix No.2

Composition	For a well /µL	For 20 wells/µL
5×Reaction Buffer	4.00	80
2mmol/L dNTPs	4.00	80
50mmol/L Manganese(II) Acetate	1.00	20
Hot Start Reverse Transcription DNA Polymerase	0.25	5
Fw & Rv Primers 2	1.00	20
TaqMan® Probe 2	0.40	8
Distilled Water, Nuclease-free	4.35	87
Total	**15.00**	**300**

3. Reaction Set Up

1. Apply 15µL of the PCR master mix No.1 and No.2 into each required well of an real-time PCR plate or an real-time PCR tube.
2. Add 5µL of the sample RNA or 5µL of the positive and negative controls(e.g. nuclease-free water).

Composition	For a well /µL
PCR master mix No.1 or No. 2	15.00
RNA sample or controls	5.00
Total	**20.00**

3. Mix the samples or controls with the PCR master mix by pipetting up and down.
4. Close the real-time PCR plate or the PCR tubes with appropriate optical adhesive film or lids.
5. Centrifuge the PCR plate in a centrifuge with a plate rotor for 30 seconds at 1,000 x g.

<Example of arrangement of applying samples in 96-well plate >

The table below is an example of 5 samples, 1 positive control, 1 negative control, N=1.

Posi:Positive control, Nega:Negative control,

S1-S5:Sample

P1: PCR Master Mix No.1, P2: PCR Master Mix No.2,

	1	2	3	4	5	6	7	8-12
A								
B		Posi P1	S1 P1	S2 P1	S3 P1	S4 P1	S5 P1	
C		Posi P2	S1 P2	S2 P2	S3 P2	S4 P2	S5 P2	
D		Nega P1						
E		Nega P2						
F								

4. Programming the Real-Time PCR instrument

For basic usage regarding the setup and programming of the different real-time PCR instruments, refer to the user manual of the respective instrument.

1. Programming

Program the following settings.

Reaction steps	Settings	
Predenature	90℃, 30 sec.	
Reverse transcription	60℃, 10 min.	
Predenature	95℃, 1 min.	
Denature	95℃, 3 sec.	45 cycles
Annealing & Extension	60℃, 5 sec.*	

*In case that a short reaction time such as 5 seconds is not programmable on an instrument, please set the shortest available time.

5. Detection

Refer to the manual of the respective instrument.

6. Reading the results

Detection of a signal within 40 cycles is interpreted as a positive.

+:Detected -:Not detected

TaqMan Probe 1	TaqMan Probe 2	Positve Control	Negative control	Interpritation
+	+	+	-	SARS-CoV-2 N gene was detected.
+	-	+	-	SARS-CoV-2 N gene was detected.
-	+	+	-	SARS-CoV-2 N gene was detected.
-	-	+	-	SARS-CoV-2 N gene was not detected .
+ or -	+ or -	+ or -	+	RNA contamination occurred. Try again.
+ or -	+ or -	-	+ or -	RNase contamination occurred or positive control was too diluted. Try again.

Code No. 281-34501（200reactions）

SARS-CoV-2 Lysis Buffer Ver.2

SARS-CoV-2 Lysis Buffer ver.2 is SARS-CoV-2-derived RNA extraction buffer from saliva or nasopharyngeal swab for real-time RT-PCR. SARS-CoV-2 can be lysed quickly and efficiently by this product which mainly contains a protease and surfactant. A simple and easy protocol with it provides reliable results, shortening time for assays and risk reduction from being infected with SARS-CoV-2.
Using SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)(Code No. 283-33209) with this product is recommended.

【Storage】

-20℃

【Precautions】

- Be careful of safety according to a guideline at your laboratory.
- Wear safety glasses and gloves during the experiment.
- Handle all components and samples in a biosafety cabinet or a clean bench.
- Be careful of RNA and RNase contamination.

【Materials required but not provided】

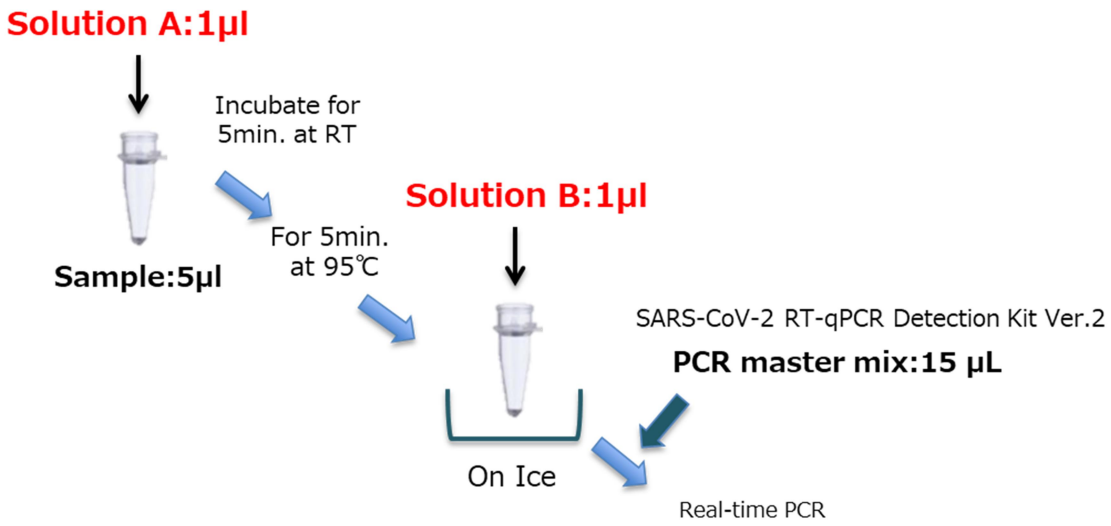
- Microcentrifuge
- Micro pipette and nuclease-free pipette tips
- Nuclease-free 1.5mL tubes*(e.g. Micro tube 1.5ml DNA LoBind, Eppendorf)

  *Low DNA/RNA binding tubes are recommended.
- Heating block(if needed)
- Ice

【Components】

Components	Volume
Solution A	1x200µL
Solution B	1x200µL

【Protocol】



1. Transfer 5µL of sample into a nuclease-free 1.5mL tube.
2. Add 1µL of Solution A into it.
3. Mix it using a vortex mixer or pipetting.*
4. Incubate it with a tube cap closed for 5 minutes at room temperature.
5. Boil it for 5 minutes at 95℃ using a heating block.
6. Cool it on ice.
7. Add 1µL of Solution B into it.
8. Mix it using a vortex mixer or pipetting.*
   The sample treatment is completed.
9. Add 15µL of the PCR master mix which prepared using SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)(Code No. 283-33209) into it.**
10. Mix it using a vortex mixer or pipetting.*
11. Transfer total volume, 22µL/tube, into a PCR tube and proceed to real-time PCR.

*Spin down a tube using a microcentrifuge after vortexing it.

**Alternatively, add 15µL of the PCR master mix which prepared using SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)(Code No. 283-33209) into a PCR tube and add 7µL of the treated sample into it.

Code No. 199-18991 (50 reactions)

For Research Use Only

SARS-CoV-2 Lysis Buffer

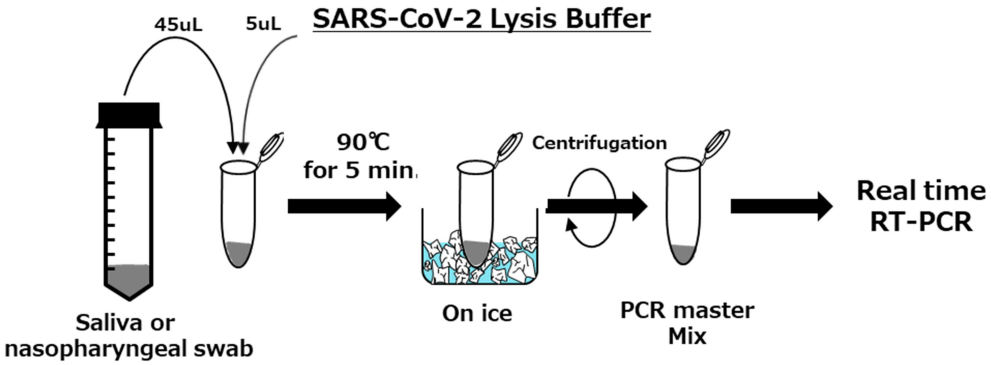
This product was discontinued. Please use the alternative product, SARS-CoV-2 Lysis Buffer ver.2(Code No. 281-34501).

SARS-CoV-2 Lysis Buffer is SARS-CoV-2-derived RNA extraction buffer from saliva or nasopharyngeal swab for real-time RT-PCR. SARS-CoV-2 can be lysed quickly and efficiently by this product which mainly contains a surfactant and reducing agent. A simple and easy protocol with it provides reliable results, shortening time for assays and risk reduction from being infected with SARS-CoV-2.
Using SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene)(Code No. 283-33209) with this product is recommended.

【Storage】
-20℃

【Precautions】
- Be careful of safety according to a guideline at your laboratory.
- Wear safety glasses and gloves during the experiment.
- Handle all components and samples in a biosafety cabinet or a clean bench.
- Be careful of RNA and RNase contamination.

【Outline of protocol】



<Preparation>

1. Thaw SARS-CoV-2 Lysis Buffer and saliva or nasopharyngeal swab samples on ice.
   If precipitation appears in SARS-CoV-2 Lysis Buffer, warm SARS-CoV-2 Lysis Buffer at 40℃ in a water bath or heating block until precipitation disappears and cool it on ice.
2. Homogenize SARS-CoV-2 Lysis Buffer by tumbling or pipetting, spin down it and cool it on ice.
   Do not vortex it to avoid bubbling.
3. Vortex samples with a vortex mixer and spin down it

<Protocol>

12. Transfer 45μL of saliva into a micro tube on ice.
13. Add 5uL of SARS-CoV-2 Lysis Buffer into it on ice.
14. Mix it with a vortex mixer and spin down it.
15. Warm it at 90℃ for 5 minutes by a heating block.
16. Cool it on ice.
17. Centrifuge it with 5,000 x g at room temperature for 1 minute.
18. Cool it and real-time PCR plates on ice.
19. Apply it as an RNA template on real-time PCR plates on ice.

Revision history

-15 November, 2020

・Volume of components of SARS-CoV-2 Real-Time RT-PCR Detection Kit(N gene) was revised.

・SARS-CoV-2 Lysis Buffer was discontinued.

・SARS-CoV-2 Lysis Buffer Ver.2 was added.

FUJIFILM Wako Laboratory Chemicals site
https://labchem-wako.fujifilm.com

FUJIFILM Wako Pure Chemical Corporation

1-2, Doshomachi 3-Chome, Chuo-Ku, Osaka 540-8605, Japan
Telephone : +81-6-6203-3741
Facsimile : +81-6-6201-5964
ffwk-cservise@fujifilm.com

FUJIFILM Wako Chemicals U.S.A. Corporation

1600 Bellwood Road
Richmond, VA 23237
U.S.A.
Telephone : +1-804-271-7677
Facsimile : +1-804-271-7791
wkuslabchem@fujifilm.com

FUJIFILM Wako Chemicals Europe GmbH

Fuggerstrasse 12
D-41468 Neuss
Germany
Telephone : +49-2131-311-0
Facsimile : +49-2131-311100
labchem_wkeu@fujifilm.com

FUJIFILM Wako Chemicals (Hong Kong) Limited

Room 1111, 11/F, International Trade Centre, 11-19 Sha Tsui Road, Tsuen Wan, N.T., Hong Kong
Telephone : + 852-2799-9019 Facsimile : + 852-2799-9808
wkhk.info@fujifilm.com

FUJIFILM Wako (Guangzhou) Trading Corporation

Room 3003, 30/F. Dong Shan Plaza 69, Xian Lie Zhong Road Guangzhou, 510095, China
Telephone : + 86-20-8732-6381 (Guangzhou) Telephone : + 86-21-6288-4751 (Shanghai)
Telephone : + 86-10-6413-6388 (Beijing)
wkgz.info@fujifilm.com