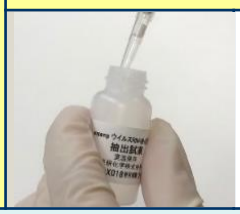
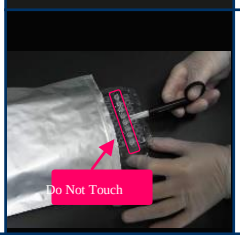
IVD CE

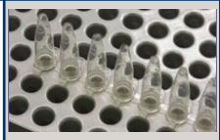
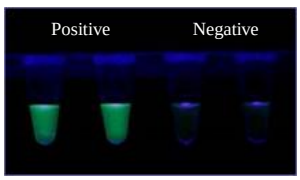
Loopamp™

Procedure for Loopamp™ SARS-CoV-2 Detection Kit【Saliva Specimen】

※ Check the Warnings and Precautions in Instructions for Use.

No.	Process	Workspace	Workflow	Remarks
No.❶	Cleaning	Biosafety cabinet Clean bench Workbench	Clean workspaces (biosafety cabinet, clean bench, and workbench) and equipment using 0.5% sodium hypochlorite solution. ◆ **Workspaces (biosafety cabinet, clean bench, workbench and etc.)** ① Spread 0.5% sodium hypochlorite solution and wait for 5 minutes. ② Wipe out liquids and wipe again with a paper towel wet with water. ③ Wipe with dry paper towel. ◆ **Equipment (micropipettes, an ice rack and etc.)** ① Wipe the surface of equipment with a paper towel wet with 0.5 % sodium hypochlorite solution and wait for 5 minutes. ② Wipe out liquids and wipe again with a paper towel wet with water. ③ Wipe with a dry paper towel.	
No.❷	Preparation of instrument	Workbench	Pre-heating: Refer to the instruction manual for the instrument. Turn on the instrument (thermal cycler / incubator / heating block with a hot bonnet) and set the temperature at 62.5℃.	**Amplification:** 62.5℃ / 35 min **Inactivation:** 80℃ / 5 min or 95℃ / 2 min
No.❸	Extraction	Biosafety cabinet 1	Transfer a saliva specimen to a laboratory. (Regarding how to collect a specimen, please check and follow your laboratory or local instruction.) Extraction Method: Option 1: Loopamp™ Viral RNA Extraction Kit: Refer to this workflow. Option 2: QIAamp Viral RNA Mini Kit or other viral RNA extraction kit that is validated by the laboratory.	Refer to the IFU enclosed in the extraction kit.
			 【Saliva specimen】 Dispense 100µL of saliva into Extraction Reagent and pipette 10 times gently to mix well and use as the sample solution. ⚠Do not open the bottle cap of the sample solution outside the biosafety cabinet. ⚠Take necessary biohazard measures.	Put on two pairs of powder-free gloves. In principle, use a sample solution immediately.
			⚠Nucleic acid extraction should be done in a biosafety cabinet. ⚠Take off the outer gloves inside the biosafety cabinet after the operation.	
No.❹	Reagent preparation	Biosafety cabinet 2	 (★) Take an aluminium pack which contains Dried RNA Amplification Reagent from a refrigerator and leave it for 5 minutes at room temperature. ⚠If the cold reaction tubes are taken out from the aluminium pack, the dried reagent may absorb moisture and deteriorate. (★) While waiting for 5 minutes, place Primer Mix., Positive Control and Negative Control on a pre-cooled ice rack for 1.5 mL tubes on ice cubes. ⚠Spin down Primer Mix., the both controls by a centrifuge for 1.5mL tubes before use and put them on the ice rack.	Operation on ice
			 Take the required number of the reaction tubes (Dried RNA Amplification Reagent) from the aluminium pack (number of sample + 2). And put them on a pre-cooled ice rack. ⚠When you take out the reaction tubes from the aluminium pack, do not touch the inside of the lid of the reaction tube. ⚠Close the aluminium pack immediately after use and put it into a refrigerator.	
No.❺	Dispense reagent and sample	Biosafety cabinet 2	 ① Dispense 15 µL of Primer Mix. into all reaction tubes. ⚠Spin down the tube of Primer Mix. by a centrifuge for 1.5 mL before use.	
		Biosafety cabinet 2	② Dispense 10 µL of a sample and close the lid of the reaction tube. ③ Dispense 10 µL of Negative Control and close the lid of the reaction tube. ④ Dispense 10 µL of Positive Control and close the lid of the reaction tube. ⚠Spin down the tube of Negative Control and Positive Control by a centrifuge for 1.5 mL before use.	Dispense Positive Control lastly.
			⚠Make sure that liquid volume in each tubes are 25 µL. It can be confirmed by the liquid height coming on the lower line. ⚠Dispense the samples and each control in a biosafety cabinet and close the lids of the reaction tubes tightly.	

Please check another side for the workflow ❶ ~ ❺.

No.	Process	Workspace	Workflow	Remarks
No.❺ (continued)	Dispense reagent and sample	Workbench	 ④ Reconstitute the dried reagents in the lids by inverting the reaction tubes and collecting the solutions in the lids. Leave the tubes standing upside down for 2 minutes to reconstitute the dried reagents. ⚠Keep 2 minutes to dissolve the dried reagent.	Use a timer for reconstituting. Until here
			 ⑤ Invert the reaction tubes for 5 times (backwards and forwards). ⑥ Spin down the reaction tubes by a centrifuge for 0.2 mL tubes.	⚠ Do Not touch the reaction tube body.
			・Since the amount of the liquid is small quantity, invert by shaking your wrist gently to transfer the liquid. ・Make sure that the dried reagents in the lids are fully dissolved. ・If there are bobbles in the reaction tubes, remove them by flicking fingers.	
No.❻	Amplification	Workbench	Amplification: 62.5 ℃ / 35 min Inactivation: 80℃ / 5 min or 95℃ / 2 min	Put the reaction tubes on the instrument. *The temperature accuracy ±0.5℃ with a hot bonnet. *Do enzyme inactivation at 80℃ for 5 minutes or 95℃ for 2 minutes after the amplification is completed. After inactivation, remove the reaction tubes from the instrument carefully.	⚠HOT CAUTION Do not touch the hot bonnet and heating block. Before removing the reaction tubes, check if the reaction tubes are closed.
No.❼	Detection	Workbench		For a valid run, the following results must be obtained: Positive Control: Green fluorescent light is emitted. Negative Control: No fluorescent light is emitted. After confirming that the run is valid, evaluate the samples as follows: Positive Sample: Green fluorescent light is emitted. Negative Sample: No fluorescent light is emitted.	*Use a blue LED Light or an UV light such as black light (wavelength 240 to 260 nm, 350 to 370 nm) for visual fluorescence detection.
No.❽	Disposal	Workbench	Do not open the tubes after amplification. Leave the lids closed and dispose of the used tubes as medical waste by incineration after double bagging with sealable plastic bags. **⚠Do not use an autoclave⚠** **⚠Do not open the lid of the reaction tubes⚠**	
No.❾	Cleaning	Biosafety cabinet Clean bench Workbench	Clean workspaces (biosafety cabinet, clean bench and workbench) and equipment you used by 0.5% sodium hypochlorite solution. Irradiate the biosafety cabinet and the clean bench after use with UV light.	Refer to No. ❶

＜Remarks＞
- The equipment used shall be dedicated to each workspace.
- Basically, use sample solution immediately after extraction (When unavoidably stored, store at 2 – 8℃ and use within 24 hours.).
- When dispensing the sample solution and each control, be sure to dispense Positive Control lastly.
  （If possible, use another micropipette only for Positive Control since it is highly concentrated.）

Sodium hypochlorite solution for cleaning

* Preparation of the sodium hypochlorite solution for cleaning:
Dilute household bleach (ex. 5% concentration) 10 times by distilled water to obtain 0.5% sodium hypochlorite

NOTE：Ventilate well when you use a sodium hypochlorite solution.

- Use 0.5% concentration of sodium hypochlorite solution for usual cleaning since a high concentration of it may damage the metal. Also, if you use it on metal, wipe up well by a wet paper towel.
- Store an undiluted sodium hypochlorite solution in a cool and dark place since it would be decomposed by light or temperature.

NOTE：Mark the date of dilution on a wash bottle. Discard it after 1 week and make a new sodium hypochlorite solution.

【Warnings and Precautions】

1. Negative results do not preclude SARS-CoV-2 infection.
2. The detection result must be judged by combination of comprehensive information such as medical examination, patient history, and epidemiological information.
3. Take necessary biohazard measures for handling infectious disease samples and refer to the latest infectious diseases' pathogen safety management regulations in your country.

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