FloodLAMP COVID-19 Tests
EasyPCR™
QuickColor™
QuickFluor™

Excerpts from DRAFT Instructions for Use

Equipment & Materials for Clinical Evaluation

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Inactivation Solution Preparation

A 100X Inactivation Solution is prepared by mixing the components in the table below and vortexing for 30 seconds. Equivalent preparations utilizing components with different source concentrations may be used such that the final 100X Concentration is achieved. Aliquots of 100X Inactivation Solution should be stored in the dark at -20°C for up to 3 months. Upon thaw, working aliquots of 100X Inactivation Solution should be stored in the dark at room temperature for up to 1 month.

100X Inactivation Solution

Component	Source Concentration	Volume (100 Samples)	Volume (2000 Samples)	100X Concentration
TCEP	0.5 M	500 µL	10 mL	250 mM
EDTA	0.5 M	200 µL	4 mL	100 mM
NaOH	10 N	117 µL	2.3 mL	1.15 N
Nuclease-free Water		183 µL	3.7 mL	
TOTAL VOLUME		**1000 µL**	**20 mL**	

Sample Preparation (wet swabs)

* For wet swab specimens (swabs in saline or unprocessed swab elution):

1) If samples are frozen, thaw unless no ice crystals are present and then keep on ice, cold block or at 4°C.
2) Pulse vortex each sample and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
3) Wipe the outside of the sample tube with 70% ethanol.

Sample Inactivation

1) Place the inactivation heater (a thermal cycler, water bath, dry heat bath or equivalent) in the BSC, turn on, and **set the temperature to hold at 100°C.**
2) **Transfer 1 mL** or available volume of each sample to an appropriately labeled 1.5 mL or 5mL tube and securely cap.
3) **Add 10µL per 1 mL sample volume of 100X Inactivation Solution, or 2µL per 0.1 mL sample volume of 50X Inactivation Solution** to each sample tube.
4) **Vortex for 30 seconds**.
5) Place sample tubes into the inactivation **heater for 8 minutes**.
6) Remove sample tubes from the inactivation heater and let **cool at room temperature for 10 minutes**.

7) Place sample tubes on ice, in the refrigerator, or on a cold block at **4°C until ready** to perform amplification reaction.
   Note: Testing of inactivated specimens **must be conducted the same day** inactivation is performed. For long term storage, keep the original specimen at ≤-70°C.

Preparing to Run Assay for the First Time

For PCR and Fluorimetric LAMP tests that use RT-PCR Instruments, complete this section of the corresponding IFU prior to continuing.

Amplification Reaction Preparation

Common to all 3 assays:

1) Place a 96-well PCR plate or PCR strip tubes onto a **cold block** or ice.
2) **Thaw frozen reagents** until ice crystals are not present.
3) Pulse vortex thawed reagents for 3 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
4) Store reagents on ice, in the refrigerator, or on a cold block at 4°C until ready to use.
5) Prepare 96-well PCR plate or PCR strip tubes with amplification reaction mix

NOTE: For Colorimetric LAMP, optionally seal 96-well PCR plate with foil seal and add sample by piercing seal.

NOTE: Ensure that positive and negative controls are included in each batch run (i.e. in each PCR plate or group of strip tubes that are heated together).

NOTE: Colorimetric LAMP Negative Control must be prepared from 0.9% Saline and 100X Inactivation Solution.

Controls

	PCR	Colorimetric LAMP	Fluorimetric LAMP
Positive Control	same, see IFU	same, see IFU	same, see IFU
Negative Control	nuclease-free water	0.9% saline with 1X Inactivation Solution	nuclease-free water
Primers (Tube Label)	PCRP (5X PCR Primer Stock)	LP (10X LAMP Primer Mix)	LP (10X LAMP Primer Mix)

PCR Amplification Reaction Preparation

1) Prepare the PCR Amplification Reaction Mix by combining the components listed in the table below.
   NOTE FOR CLINICAL EVAL: Use a 5 mL tube to accommodate the SUBTOTAL VOLUME of 2300 µL.
   NOTE: Component volumes should be scaled proportionally for the number of reactions.
2) Vortex the PCR Amplification Reaction Solution for 10 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
3) Add 18 µL of the PCR Amplification Reaction Solution into the wells of the PCR plate or PCR strip tubes.

PCR Amplification Reaction Mix

Component	Volume (1 reaction)	Volume (1 reaction x 100) 1 x 96-plate w/ 4% overage
5X PCR Primer Stock	4 µL	400 µL
Nuclease-free Water	3 µL	300 µL
PCR Master Mix	10 µL	1000 µL
PCR RT	1 µL	100 µL
SUBTOTAL VOLUME	**18 µL**	**1800 µL**
Sample	2 µL	
REACTION VOLUME	**20 µL**	

Sample Addition

NOTE: Ensure that positive and negative controls are included in each batch run (i.e. in each PCR plate or group of strip tubes that are heated together).

1) Add 2 uL of each sample into a separate tube in the amplification reaction PCR plate or strip tubes.
2) Mix by pipetting.
3) If using PCR plate optical seal (optionally using heat sealer). If using PCR strip tubes, cap strips.
4) Pulse vortex and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.

Continue to section “Run the Assay” on pg. 20 of FloodLAMP EasyPCR™ COVID-19 Test IFU.

Colorimetric LAMP Amplification Reaction

1) Combine components of Primer-Guanidine Solution per volumes listed in Table 7, or proportionally scaled for the number of reactions to be run.
   NOTE FOR CLINICAL EVAL: Use a 5 mL tube to accommodate the SUBTOTAL VOLUME of 2300 µL. The Colorimetric LAMP MM should be added to the tube the Primer-Guanidine Solution is prepared in.
   NOTE: Component volumes should be scaled proportionally for the number of reactions.
   NOTE: The Primer-Guanidine Solution may be prepared in advance and stored at -20°C for up to 1 month.
2) Pulse vortex and briefly spin down in a centrifuge.
3) Prepare the Colorimetric LAMP Amplification Reaction Mix by adding the Colorimetric LAMP MM to the Primer-Guanidine Solution per the volumes listed in Table 8.
4) Vortex the Colorimetric LAMP Amplification Reaction Solution by for 10 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
5) Add 23 µL of the Colorimetric LAMP Amplification Reaction Solution into the wells of the PCR plate or PCR strip tubes.
   NOTE: Reaction plates/strip tubes comprising the Colorimetric LAMP Amplification Reaction Solution may be prepared in advance, capped/sealed, and stored at -20°C for up to 3 days prior to addition of the sample.

Primer-Guanidine Solution

Component	Volume (1 reaction)	Volume (1 reaction x 100) 1 x 96-plate w/ 4% overage
10X LAMP Primer Mix	2.5 µL	250 µL
Guanidine HCl (400 mM)	2.5 µL	
Guanidine HCl (6 M)		16.7 µL
Nuclease-free Water	5.5 µL	783 µL
TOTAL VOLUME	**10.5 µL**	**1050 µL**

Colorimetric LAMP Amplification Reaction

Component	Volume (1 reaction)	Volume (1 reaction x 100) 1 x 96-plate w/ 4% overage
Primer-Guanidine Solution	10.5 µL	1050 µL
Colorimetric LAMP MM	12.5 µL	1250 µL
SUBTOTAL VOLUME	**23 µL**	**2300 µL**
Sample	2 µL	
REACTION VOLUME	**25 µL**	

Sample Addition and Heating

NOTE: Ensure that positive and negative controls are included in each batch run (i.e. in each PCR plate or group of strip tubes that are heated together).

1) Turn on the amplification heater (a thermal cycler, water bath, dry heat bath or equivalent) and set the temperature to hold at 65°C.
   NOTE: Amplification heater should be located in a separate, dedicated BSC or area of the lab. Proper cross contamination prevention practices are required, such as glove changes, to prevent amplicon contamination.
2) Add 2 µL of each sample into a separate tube in the amplification reaction PCR plate or strip tubes.
3) Mix by pipetting.
4) If using PCR plate, seal with foil seal, optical seal (optionally using heat sealer). If using PCR strip tubes, cap strips.
5) Pulse vortex and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
6) Place the plate or strip tubes in the heater and set timer for 25 minutes.
7) Remove the plate or strip tubes from the heater after 25 minutes.
8) Let cool for 1 minute and then interpret the test results.

Continue to section "Test Controls" on pg. 17 of FloodLAMP QuickColor™ COVID-19 Test IFU.

Fluorimetric LAMP Amplification Reaction Preparation

1) Prepare the Fluorimetric LAMP Amplification Reaction Mix by combining the components listed in the table below.
2) Vortex the Colorimetric LAMP Amplification Reaction Solution for 10 seconds and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.
3) Add 23 µL of the Fluorimetric LAMP Amplification Reaction Solution into the wells of the PCR plate or PCR strip tubes.

Fluorimetric LAMP Amplification Reaction

Component	Volume (1 reaction)	Volume (1 reaction x 100) 1 x 96-plate w/ 4% overage
10X LAMP Primer Mix	2.5 µL	250 µL
Nuclease-Free Water	7.5 µL	750 µL
Fluorimetric LAMP MM	12.5 µL	1250 µL
Fluorescent Dye	.5 µL	50 µL
SUBTOTAL VOLUME	**23 µL**	**2300 µL**
Sample	2 µL	
REACTION VOLUME	**25 µL**	

Sample Addition

NOTE: Ensure that positive and negative controls are included in each batch run (i.e. in each PCR plate or group of strip tubes that are heated together).

1) Add 2 uL of each sample into a separate tube in the amplification reaction PCR plate or strip tubes.
2) Mix by pipetting.
3) If using PCR plate optical seal (optionally using heat sealer). If using PCR strip tubes, cap strips.
4) Pulse vortex and briefly spin down in a centrifuge to collect the liquid at the bottom of the tube.

Continue to section “Run the Assay” on pg. 22 of FloodLAMP QuickFluor™ COVID-19 Test IFU.

Contact

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Equipment & Materials for Clinical Evaluation

Equipment Provided by Lab
Thermal Cycler - set at 65ºC for LAMP Amplification Reaction (do not use heated lid)
BioRad CFX96 RT-PCR Instrument

Equipment Provided by FloodLAMP
Water Bath backup - set 99.9ºC for Inactivation, included rack for tubes

Reagents Provided by FloodLAMP (room temp)
0.9% Saline Solution - in 1.5 mL screw tube labeled "Saline", for creating Color LAMP neg control by adding 1/100th volume of 100X Inactivation Solution
TCEP (.5M) - glass ampule vial
EDTA (.5M) - in 1.5 mL screw tube labeled "EDTA"
NaOH (10N) - in 1.5 mL screw tube labeled "NaOH"
Guanidine HCl (6M) - in 1.5 mL screw tube labeled "GUD"
Nuclease-free Water - in 1.5 mL screw tube labeled "dH20"

Reagents Provided by FloodLAMP (4ºC/cold pack)
Positive Control - in single PCR tube
5X PCR Primer Stock - in 1.5 mL screw tube labeled "PCRP"
10X LAMP Primer Mix - in 2 x 1.5 mL screw tube labeled "LP"
Colorimetric LAMP MM - in 1.5 mL screw (NEB source tube, M1804)
Fluorimetric LAMP MM - in 1.5 mL screw (NEB source tube, E1700)
Fluorescent Dye - in 1.5 mL screw (NEB source tube, E1700)
PCR Master Mix - in 1.5 mL screw (NEB source tube, E3006)
PCR RT - in 1.5 mL screw (1.5mL screw tube labeled “RT”

Consumables Provided by FloodLAMP
5 mL transport tubes - MTCBio [100] for samples
1.5 mL tubes [5] spare
5 mL snap cap tubes [3] for Master Mix preparation
PCR Plates - Eppendorf [1] for Colorimetric LAMP
PCR Plates - Applied Bio [2]for PCR and Fluorimetric LAMP
Optical Plate Seals - ABI [3]for PCR and Fluorimetric LAMP
Foil Plate Seals - Excel [2] for Colorimetric LAMP

Miscellaneous Provided by FloodLAMP
Separate Flipper Racks (5 orange)
PCR cold blocks

Notes and Suggestions