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## CRISPR-Enhance Lateral Flow Assay

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We are still developing and optimizing this protocol

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## Abstract

The CRISPR-Enhance SARS-CoV-2 detection kit has been designed to detect fragments of the Nucleocapsid ("N") gene and Envelope gene (E) of SARS-CoV-2. An included third target is the human RNase P POP7 gene ("RP") which serves as a control for the extraction of the clinical sample in the absence of a positive SARS-CoV-2 result. The entire reaction from RT-LAMP amplification to CRISPR-based detection of the target analytes can be performed in approximately one hour.


The CRISPR-Enhance kit comprises of two steps. Step one is a reverse transcriptase loop-mediated amplification (RT-LAMP) where targeted SARS-CoV-2 genomic RNA is reverse transcribed to DNA, and this DNA is amplified by a strand-displacing DNA polymerase. Step two is the transcription of the amplified DNA to activate the collateral cleavage activity of a CRISPR complex programmed to the target RNA sequence. This collateral activity results in cleavage of nucleic acid reporters, resulting in a visual readout using a paper strip in approximately 2 mins.


## Guidelines

All procedures should be performed in a BSL2 laboratory, and specimens should be handled within a Biological Safety Cabinet. All necessary safety precautions should be taken according to the Laboratory guidelines. Precautions must also be taken to prevent cross-contamination of samples.


## Materials


### MATERIALS

 QuickExtract™ RNA Extraction Kit **Lucigen Catalog #QER090150**


 WarmStart®Colorimetric LAMP 2X Master Mix with UDG (Cat.No. M1804S) **New England Biolabs Catalog #M1804S**


### STEP MATERIALS


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## Protocol materials


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## Troubleshooting

## Safety warnings

-  1. Handle all infectious samples with appropriate CDC approved methods
- 2. Wear appropriate PPE such as lab coats, gloves, N95 respirators, safety goggles etc when handling infectious samples
- 3. Discard all biohazard waste appropriately
- 4. Clean all work surfaces with bleach and IPA after use


## Nucleic Acid Extraction



18m

1


18m


- The CRISPR-Enhance SARS-CoV-2 detection kit uses QuickExtract™ RNA Extraction Kit

 QuickExtract™ RNA Extraction Kit **Lucigen Catalog #QER090150**

- Add  10 µL of patient sample to  10 µL of pre-aliquoted QuickExtract solution.
- Heat the above mixture at  65 °C for  00:15:00 followed by  98 °C for  00:03:00 .

## RT-LAMP Master Mix Preparation

- 2
- Label a new  1.5 mL microcentrifuge tube for each target ( N, E and RP) and prepare a RT-LAMP Master Mix consisting of the WarmStart®Colorimetric LAMP 2X Master Mix with UDG.

 WarmStart®Colorimetric LAMP 2X Master Mix with UDG (Cat.No. M1804S) **New England Biolabs Catalog #M1804S**







and the appropriate 10x Primer Mix using the recipe in Table 1 below. Make enough of each master mix for all samples to be tested and the necessary controls for each run.

	Reagent Name	Volume per reaction	Total Volume
	WarmStart®Colorimetric LAMP 2X Master Mix with UDG	12.5 µL	12.5 µL x (N+1)
	10x Primer Mix (N, E or RP)	2.5 µL	2.5 µL x (N+1)
	RNAse-Free Water	5 µL	5 µL x (N+1)
	<b>Total Volume</b>	<b>20 µL</b>	<b>20 µL x (N+1)</b>

**Table 1: Target Specific RT-LAMP Master Mix Recipe**

N = number of extracted samples plus number of controls. Prepare enough for 1 extra (N + 1) sample to allow for overage during reaction set-up.

## RT-LAMP Amplification







- 3
  - Label a strip tube (  0.2 mL ) with the target name (e.g. N) and strip number corresponding to each sample.
  - Add  20 µL of the RT-LAMP Master Mix from the previous step into one well for each sample and control to be amplified. Repeat for the remaining 2 targets using a new strip for each target (e.g. E or RP)
  - Add  5 µL of extracted RNA in each respective strip tube containing the RT-LAMP Master mix. Vortex the strip tube for  00:00:03 and spin down for  00:00:03 in microcentrifuge with a  0.2 mL tube adaptor.

Reagent	Volume per reaction
RT-LAMP Master Mix	20 µL
RNA Sample or Controls	5 µL
<b>Total Volume</b>	<b>25 µL</b>

**Table 2: RT-LAMP Assay Components and reaction volume**

- Heat the mixture at  65 °C for  00:40:00


## CRISPR-Cas Reaction Preparation

- 4
  - Preheat a fluorescence microplate reader to  37 °C .
  - For each target tested label a  1.5 mL tube with the target name (e.g. N, E or RNASE-P) and "Cas Mix". Prepare a CRISPR Cas Master Mix using the following recipe in Table 3 below, scaling as required for the number of assays to be run (one Cas assay for every RT-LAMP reaction).
  - Incubate the mixture at  37 °C for  00:15:00
  - Pulse vortex for  00:00:03 and spin down for  00:00:03 in a microcentrifuge after all components are added.

	Reagent Name	Volume per Reaction	Volume Total
	NEB 2.1 Buffer	1.2 $\mu\text{L}$	$1.2 \mu\text{L} \times (N+1)$
	3 $\mu\text{M}$ crRNA (N or E or RP)	0.8 $\mu\text{L}$	$0.8 \mu\text{L} \times (N+1)$
	3 $\mu\text{M}$ IbCas12a	0.4 $\mu\text{L}$	$0.4 \mu\text{L} \times (N+1)$
	RNAse-Free Water	9.6 $\mu\text{L}$	$9.6 \mu\text{L} \times (N+1)$
	Total Volume	12 $\mu\text{L}$	$12 \mu\text{L} \times (N+1)$

Table 3: Target CRISPR Cas Master Mix Recipe

*N* = number of extracted samples plus number of controls. Prepare enough for 1 extra (*N* + 1) sample to allow for overage during reaction set-up.

- In a separate  1.5 mL microcentrifuge tube prepare the FAM-Biotin reporter as per Table 4

	Reagent Name	Volume per Reaction	Volume Total
	100 $\mu\text{M}$ FAM-Biotin Reporter	0.2 $\mu\text{L}$	$0.2 \mu\text{L} \times (N^* + 1)$
	RNAse-Free Water	25.8 $\mu\text{L}$	$25.8 \mu\text{L} \times (N^* + 1)$
	Total Volume	12 $\mu\text{L}$	$12 \mu\text{L} \times$





			(N* +1)
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Table 4: Fluorescence Reporter Mix Recipe

*N\* = number of extracted samples multiplied by the total number of genes plus number of controls. Prepare enough for 1 extra (N\* + 1) sample to allow for overage during reaction set-up*

## CRISPR-Cas Detection

- 5
  - For each sample, mix 12 µL of the CRISPR-Cas Master mix with 26 µL of the Reporter in each well of a 0.2 mL strip tube.
  - Add 2 µL of the RT-LAMP amplicon to the well in the strip tube containing the appropriate IbaCas12a-crRNA complex.
  - Incubate the mixture at  37 °C for  00:10:00 to allow for sufficient trans-cleavage activity
  - Add a Milenia HybriDetect (TwistDx) lateral flow strip and visualise the result after approximately 2 min
  - A single band, close to the sample application pad indicates a negative result, whereas a single band close to the top of the strip or two bands indicates a positive result