SARS-CoV-2 (COVID-19) Colorimetric LAMP

New England Biolabs – Nathan Tanner, 10 March 2020

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Materials

Material	Concentration	Lot
NEB M1800L LAMP Mix	2X	10069263
CoV-2 Gene N LAMP Primer Mix	25X	YZ200310

Protocol

1) Thaw LAMP Master Mix and LAMP Primer Mix at room temperature
2) Mix thoroughly by vortexing or inversion, ensure that any precipitation in the M1800 mix is resuspended
3) Assemble reactions using the following recipe:

Material	Concentration	Volume (per 25 uL Reaction)
M1800 LAMP Mix	2X	12.5 uL
LAMP Primer Mix	25X	1 uL
Sample	-	As desired
Water	-	To 25 uL

SAMPLE VOLUME NOTE: we recommend extracted nucleic acid to be eluted or prepared in water to avoid carrying over excess buffer to the reaction. Material eluted in TE or similar elution buffer should be kept to less than 5 uL (20% v/v) of the final reaction volume. To increase sample volume, use 0.1X Elution Buffer or water for preparation of the nucleic acid.

4) Verify reactions are pink; if yellow, repeat with lower sample volume or sample adjusted to pH ~8.
5) Place reactions at 65 °C, incubate for 30 minutes. *NOTE:* For high copy number samples, time can be shortened to 15–20 minutes. For very low copy samples or maximum sensitivity, extend time to 40 minutes.
6) Inspect reaction tubes for yellow color. Allow reactions to cool from 65°C by placing at room temperature for improved color contrast, or place tubes on ice for 5 seconds before inspecting.
7) Discard completed reactions without opening reaction vessels.

Primer Sequences (5′-3′)

GeneN-F3	TGGCTACTACCGAAGAGCT
GeneN-B3	TGCAGCATTGTTAGCAGGAT
GeneN-FIP	TCTGGCCCAGTTCCTAGGTAGTCCAGACGAATTCGTGGTGG
GeneN-BIP	AGACGGCATCATATGGGTTGCACGGGTGCCAATGTGATCT
GeneN-LoopF	GGACTGAGATCTTTCATTTTACCGT
GeneN-LoopB	ACTGAGGGAGCCTTGAATACA