EZassay

RT-LAMP-CRISPR RNA detection kit (one-step)

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Catalog Code: RT-LAM-CAS-01
RT-LAM-CAS-10

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Product Introduction

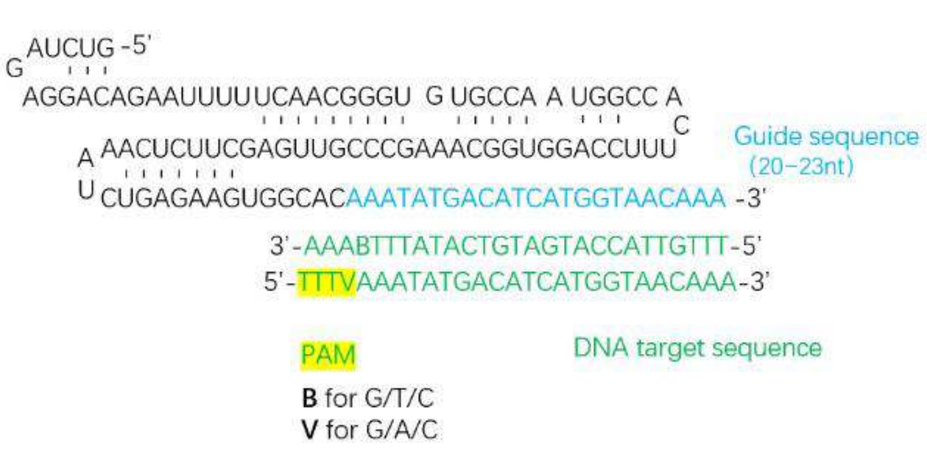
This product combines RT-LAMP and CRISPR/Cas12b to achieve sensitive and specific RNA detection. All reactions are performed in one tube and in one step. It makes R&D of point-of-care products easily.

Materials supplied

Component	RT-LAM-CAS-01 96T	RT-LAM-CAS-10 96T*10
Reaction Buffer (2X)	1.5ml	15 ml
RT-LAMP Enzyme Mix (25X)	100 µl	1000 µl
Cas12b Protein (10µM)	80 µL	800µl
Reporter (20µM)	130 µl	1300 µl
Positive Control	50 µl	500 µl

Required materials

i. Template RNA
ii. LAMP primers (online software：http://primerexplorer.jp/e/)
iii. Q-PCR machine or heat block
iv. Nuclease-free water
v. sgRNA for AapCas12b. Recommend High Yield crRNA/sgRNA Synthesis Kit (Cat.: SG-RNA-001）
(AapCas12b crRNA scaffold sequence: 5’ -
GUCUAGAGGACAGAAUUUUUCAACGGGUGUGCCAAUGGCCACUUUCCAGGUGGCAAAGCCCG
UUGAGCUUCUCAAAUCUGAGAAGUGGCAC -3’



Storage

-20°C. Suggest aliquot. Avoid repeated freeze-thaw.

Preparation of reagents

1. Mix the reagents after melting on ice.
2. Set Q-PCR working temperature at 60 °C. Turn off the lid heating function or set to 60 °C.
3. Prepare reactions as described in the table below.

Component	Volume	Working concentration
Reaction Buffer (2X)	12.5μl	1X
RT-LAMP Enzyme Mix (25X)	1μl	1X
Cas12b Protein (10μM)	0.75μL	300nM
Primers (10X)	1.25μL	1.6 μM FIP/BIP, 0.4 μM LF/LB, 0.2 μM F3/B3,
Reporter (20μM)	1.25μL	1μM
sgRNA (25μM)	1μL	1μM
Template DNA*	1μL	>10 copies or more
ddH2O	Up to 25μL	-

* For no template control group, use Nuclease-free water.
For positive control group, add 5μL Positive control that provided in the kit.
Template, primers, and probe with FAM label are already mixed as positive control.

4. Read fluorescence in Q-PCR machine for 30 to 60 minutes at 60°C.

Notes

・Designate and use distinct areas for sample preparation, reaction setup, and analysis to avoid carry-over contamination.
・It is recommended to add a negative control without template for each experiment.
・To guarantee good reproducibility, it is recommended that the template RNA be added last.
・If Q-PCR machine is used, please make sure to turn off the heat lid function or set to 60 ℃.
・RNase inhibitor (Cat. MRI-2000) and RNase-free consumables are recommended for RNA experiment.
・The optimal concentration of sgRNA and reporter varies depending on the projects.

Nucleic acid detection based on CRISPR/Cas technology diagram