E. coli qLAMP detection Kit

Real-time quantitative analysis of the E. coli gene region eae

Catalog Number: NA2059-p

For Research Use Only. Not for use in Diagnostic Procedures.

1. Background

E. coli (Escherichia coli) – A common bacterium in human/animal intestines; most strains are harmless, but some are pathogenic.

Diarrheagenic E. coli – Pathogenic strains causing diarrhea. Major types:

- **EPEC (Enteropathogenic E. coli)** – Attaches to intestine via intimin, encoded by **eae** gene.
- **STEC (Shiga toxin-producing E. coli)** – Produces Shiga toxin (encoded by *stx* genes).
- **EHEC (Enterohemorrhagic E. coli)** – Subset of STEC that also carries **eae** (e.g., O157:H7).

The *eae* gene – Encodes **intimin**, an outer membrane protein that enables tight attachment to intestinal epithelial cells, causing "attaching and effacing" lesions.

Presence of *eae* helps differentiate EPEC/EHEC from other diarrheagenic E. coli.

Detection significance – Identifying *eae* (and *stx*) allows surveillance of pathogenic E. coli in clinical, food, and environmental samples (e.g., wastewater).

2. Test Principle

Attogene's LAMP kit for E. coli is designed for the In vitro analysis of the crucial genetic marker eae. The eae gene specific primer and probe mix is provided to be detected through the FAM channel on a LAMP machine. A sample is obtained and washed to extract a clean gDNA sample. A reaction mixture is assembled from primers, probe, master mix, and gDNA samples as required. The LAMP machine of choice is set up and loaded as needed and the mixture undergoes PCR amplification. The Primer mix provided exploits the Bst polymerase to amplify the gene region of interest; while the DNA probe mixture is cleaved during amplification to release a FAM fluorophore. The resulting FAM release can be detected on a variety of LAMP platforms

3. Applications

- This kit can be used for specific analysis of the eae gene region in liquid gDNA samples.

4. Equipment and Reagents Needed (not provided)

- Real-time LAMP Instrument
- LAMP 2X Master Mix
- DNA extraction kit/ gDNA sample
- LAMP reaction tubes/plate
- Vortex and centrifuge
- PCR clean 1mL tube
- Micropipettes & Tips

5. Components Provided in This Kit

- 170ul eae region specific primer pairs (150 reactions)
- 170ul eae region specific fluorescent probe (150 reactions)
- 1.5ml PCR clean water

6. Reagents Preparation

Master Mix (not Provided)

-Caution this reagent is sensitive to contamination and should only be handled in a clean area away from positive control template.

-Store at -20C. Master Mix is stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required aliquots of Master Mix should be made and stored at -20C to minimize freeze thaw and contamination risk.

Primer/ Probe Mixture

-Caution these reagents are sensitive to contamination and should only be handled in a clean area away from positive samples.

-Store at -20C. Primer/Probe is stable when stored at -20C. Freeze and thaw cycles should be minimized to increase shelf life.

-If required aliquots of Primer/Probe should be made and stored at -20C in the dark to minimize freeze thaw and contamination risk.

7. Control Preparation

Negative extraction control (NEC)

-If necessary, prepare one NEC each time extracting DNA from your sample.

-RNase/DNase free water is used in place of sample in the extraction system to create a negative for the DNA isolation method.

- The NEC will serve as a contamination control method for the isolation.

No Template control (NTC)

-If necessary, a NTC can made by replacing gDNA in the LAMP reaction with RNase/DNase free water

-The NTC is used to check for contamination during LAMP plate set up

8. Assay Set Up

-gDNA isolation will need to be done before starting an experiment. For optimal results use >10ng/uL of gDNA with a ratio of >1.80 in your experiment. IEC multiplexing can also be done to ensure proper DNA extraction (not included).

-Plate set up will vary with the quantity of samples you need to run on your plate. A NEC is preferably included in each plate set up. NTCs should be included in each plate set up.

-Determine the number of reactions to set up in your assay (including NEC, PCT and any NTCs for your plate). It is necessary to make extra reaction mixture to allow for pipetting error.

-For convenience a large solution of LAMP components will be mixed shortly before starting a reaction and subsequently aliquoted into your plate or tubes. Each LAMP run will use 19uL of this reaction mixture and 1ul of isolated gDNA/NTC/PCT/NEC based on the experiment set up.

9. LAMP detection protocol

1. For each DNA sample prepare a reaction mix according to the table below:
(Include sufficient reactions for positive and negative controls)

Reagent	Quantity
2X LAMP Master Mix	10uL
PCR Water	7uL
Eae Probe	1uL
Eae Primer set	1uL
Final Volume	19uL

2. Pipette 19uL of this mixture into each well according to your LAMP experimental plate set up.

3. Pipette 1uL of sample gDNA into each well, according to your experiment. For negative controls replace the gDNA sample with 1uL of RNase/DNase free water to bring the total volume to 20uL.

10. LAMP Amplification Protocol

Amplification conditions using 2x LAMP Master Mix:

Time	Temperature	Detection Format
45 min.	65C	eae = FAM (456-510)

11. Expected Performance

Before Interpreting results, it is necessary to verify the integrity of the reaction. If the following criteria are not satisfied, then testing needs to be repeated.

a. NEC is free from amplification in the FAM (465-510) channel.

b. NTC is free from amplification in the FAM (465-510) channel.

Manually inspect amplification criteria are fulfilled for all samples to verify the integrity of the results.

12. Interpreting the test results

If all the data analysis criteria are fulfilled, then each sample can be assessed with the following metric:

Target	Positive control	Negative control	Result
+	+	-	Positive Quantitative result (calculate quantity)
-	+	-	Negative result
+/-	+	Tt<35	Experiment failed (contamination)
+/-	+	Tt>35	*
+/-	-	+/-	Experiment failed

*The sample must be reinterpreted based on relative signal of the Target vs. Negative control.

13. General Instructions

13.1 Shaking of Reagents

- Shake each reagent gently before use.

13.2 Out of Date Kits

- Don't use kits that are expired. Don't exchange the reagents of different batches, or else it will drop the sensitivity.

14. Storage

- Storage condition: -20°C
- Storage period: 12 months

Customer Notes:

Contact Us

Attogene

3913 Todd Lane, Suite 310 Austin, TX 78744

Phone: 512-333-1330

Email: sales@attogene.com

Web: www.attogene.com