Escherichia coli NanoLuc®

BAA-2584-PACK™

Description

A kit containing reporter-labeled *Escherichia coli* designed to distinguish control strain cross-contamination from true contamination in test samples. This product is appropriate for use as a positive control in quality control assays for *E. coli*.

Type strain: No
Serotype: O121:H19
Toxigenic: Yes
Toxin genes: *eae* (Intimin) positive; *stx1* (Shiga toxin 1) negative; *stx2* (Shiga toxin 2) positive

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your

responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Medium:
ATCC Medium 18: Trypticase Soy Agar/Broth
Temperature: 37°C
Atmosphere: Aerobic

Handling Procedures

Kit Components (9 Reactions):

Component	Format	Volume	Storage
Escherichia coli NanoLuc®	Frozen in glycerol	3 vials	-80°C or colder
Nano-Glo® Luciferase Assay Buffer	Frozen in glycerol	490 µL	-20°C or colder
Nano-Glo® Luciferase Assay Substrate	Frozen in glycerol	12 µL	-20°C or colder

Equipment and Materials Required but not Supplied:

Strain Propagation and Detection	
Trypticase Soy Agar/Broth	Inoculation loop
Water bath	Eppendorf tubes
70% Ethanol	Cotton swab
37°C incubator	Laboratory paper (Whatman)
Pipette and tips	Centrifuge

1. Prepare a sterile test tube that contains #18 broth (4 to 5 mL).
2. Thaw the sample in a 30°C to 35°C water bath, until just thawed (approximately 5 minutes). Immerse the ampoule just sufficient to cover the frozen material. Do not agitate the ampoule.
3. Immediately after thawing, wipe down the ampoule with 70% ethanol and transfer the suspension into the broth tube. Mix well.
4. Incubate the culture at 37°C for 24 hours.
5. Use the culture as a control in assays for *E. coli*.

Detection of Control Strain

Swab Method:

1. Prepare the reaction solution in an Eppendorf tube by mixing 1 µL of substrate with 49 µL of buffer.
2. Collect several colonies with a cotton swab.
3. Saturate the cotton swab with the prepared reaction solution.
4. Visualize reporter expression in a dark area.

Paper Method:

1. Prepare the reaction solution in an Eppendorf tube by mixing 1 µL of substrate with 49 µL of buffer.
2. Saturate laboratory paper with the reaction solution.
3. Collect several colonies onto an inoculation loop.

4. Scratch colonies on the saturated paper.
5. Visualize reporter expression in a dark area.

Broth Method:

1. Prepare the reaction solution in an Eppendorf tube by mixing 1 µL of substrate with 49 µL of buffer.
2. Centrifuge 500 µL of broth culture. Remove the supernatant.
3. Resuspend the pellet in the reaction solution.
4. Visualize reporter expression in a dark area.

Troubleshooting

Problem: The control strain does not luminesce after the addition of the reaction solution

Cause	Solution
Over-passage of the strain, resulting in loss of the plasmid.	Do not passage the strain more than 2 times.
The reaction solution is old or improperly prepared.	Discard. Prepare reaction solution with ≥ 5% substrate and 95% buffer. Mix well.
Inadequate amount of reaction solution used.	Ensure that your sample is fully saturated.
Contamination with another strain.	Discard the culture. Begin with a fresh culture.

Problem: The frozen culture cannot be recovered

Cause	Solution
Culture is not growing due to improper culture conditions.	Check that the culture conditions (medium, temperature, and atmosphere) are appropriate. View culture periodically to check the condition. Increase time in culture after recovery.

Safety

See the appropriate Material Safety Data Sheets regarding safety precautions for the components of this product.

Notes

The Reporter-Labeled Control Strain Kit is stable at -80°C until the printed expiration date, provided there is no contamination. Care should be taken to minimize the number of freeze-thaw cycles when handling strains and reagents as it may affect the quality and shelf-life of the product. For best results, prepare fresh reaction solution by mixing the buffer and substrate before each use.
This strain was confirmed by PCR to carry the *stx*2 and *eaeA* genes (*stx*1-, *stx*2+, *eaeA* +).
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Additional information on this culture is available on the ATCC® web site at www.atcc.org.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Escherichia coli* NanoLuc® (ATCC BAA-2584-PACK)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

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