



***Escherichia coli* NanoLuc[®]**

BAA-2582-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: O103:H111

Toxigenic: Yes

Toxin genes: *eae* (Intimin) positive; *stx1* (Shiga toxin 1) positive; *stx2* (Shiga toxin 2) negative

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

BAA-2582-PACK

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):

BAA-2582-PACK

Component	Format	Volume	Storage
<i>Escherichia coli</i> 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.

BAA-2582-PACK

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

BAA-2582-PACK

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 *stx1* and *eaeA* genes (*stx1+*, *stx2-*, *eaeA+*).

NanoLuc[®] and Nano-Glo[®] are registered trademarks of Promega Corporation. NanoLuc[®] and Nano-Glo[®] Technologies are licensed from Promega Corporation. 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-2582-PACK)

References

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

Warranty

The product is provided 'AS IS' and the viability of ATCC[®] products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory

BAA-2582-PACK

results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP standards, typicality, safety, accuracy, and/or noninfringement.

Disclaimers

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. Any proposed commercial use is prohibited without a license from ATCC.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate or complete and the customer bears the sole responsibility of confirming the accuracy and completeness of any such information.

This product is sent on the condition that the customer is responsible for and assumes all risk and responsibility in connection with the receipt, handling, storage, disposal, and use of the ATCC product including without limitation taking all appropriate safety and handling precautions to minimize health or environmental risk. As a condition of receiving the material, the customer agrees that any activity undertaken with the ATCC product and any progeny or modifications will be conducted in compliance with all applicable laws, regulations, and guidelines. This product is provided 'AS IS' with no representations or warranties whatsoever except as expressly set forth herein and in no event shall ATCC, its parents, subsidiaries, directors, officers, agents, employees, assigns, successors, and affiliates be liable for indirect, special, incidental, or consequential damages of any kind in connection with or arising out of the customer's use of the product. While

BAA-2582-PACK

reasonable effort is made to ensure authenticity and reliability of materials on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of such materials.

Please see the material transfer agreement (MTA) for further details regarding the use of this product. The MTA is available at www.atcc.org.

Copyright and Trademark Information

© ATCC 2023. All rights reserved.

ATCC is a registered trademark of the American Type Culture Collection.

Revision

This information on this document was last updated on 2022-10-22

Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor