# Escherichia coli NanoLuc

## **BAA-2581-PACK**<sup>™</sup>

## 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:** 045:H2 **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-2581-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):



## Escherichia coli NanoLuc<sup>®</sup>

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Component	Format	Volume	Storage
<i>Escherichia coli</i> NanoLuc <sup>®</sup>	Frozen in	3 vials	-80°C or
	glycerol		colder
Nano-Glo <sup>®</sup> Luciferase Assay Buffer	Frozen in	490 µL	-20°C or
	glycerol		colder
Nano-Glo <sup>®</sup> Luciferase Assay	Frozen in	12 µL	-20°C or
Substrate	glycerol	τς μι	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).
- 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  $\mu L$  of substrate with 49  $\mu L$  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  $\mu L$  of substrate with 49  $\mu L$  of buffer.
- 2. Saturate laboratory paper with the reaction solution.
- 3. Collect several colonies onto an inoculation loop.

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- 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  $\mu L$  of substrate with 49  $\mu L$  of buffer.
- 2. Centrifuge 500  $\mu L$  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,	Do not passage the strain more than 2	
resulting in loss of the plasmid.	times.	
The reaction solution is old or	Discard. Prepare reaction solution with $\ge 5\%$	
improperly prepared.	substrate and 95% buffer. Mix well.	
Inadequate amount of reaction	Ensure that your sample is fully saturated.	
solution used.		
Contamination with another strain.	Discard the culture. Begin with a fresh	
contamination with another strain.	culture.	

#### **Problem:** The frozen culture cannot be recovered

Cause	Solution
Culture is not growing due to improper	Check that the culture conditions (medium,
	temperature, and atmosphere) are appropriate. View
culture conditions.	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.



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### 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*1 and *eaeA* genes (*stx*1+, *stx*2-, *eaeA* +).

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## **Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Escherichia coli* NanoLuc<sup>®</sup> (ATCC BAA-2581-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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