




Product Sheet


pNK2887 (ATCC® 77343™)

Escherichia coli

Please read this FIRST



Storage Temp.
**Store unopened
freeze dried vial
at 4°C.**



Biosafety Level
1

Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Escherichia coli* (ATCC® 77343™)

Shipping Information

Freeze dried *Escherichia coli* HB101 containing the plasmid.

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

This construct is useful for transposon mutagenesis in *Escherichia coli*. It also permits generation of promoter fusions of target genes to Plac-UV5, if the transposon inserts in the correct orientation. It contains the *ats1 ats2* transposase gene that permits relaxed insertion specificity (altered target specificity, ATS). Expression is regulated by the Ptac promoter and is inducible by IPTG. The transposase segment extends from IS10R to the EcoRI site at nt 3140 of Tn10, with a deletion of nucleotides 1329-1942 to remove the transposase binding site. The construct also contains a 1.9 kb mini-Tn10 cassette conferring kanamycin resistance, bounded by inverted repeats of the outermost 70 bp of IS10R and embedded in 40 bp of lambdacl₁ terminating in HindIII sites. It differs from pNK2859 (ATCC 77338) by also carrying a Plac-UV5 promoter in a BamHI fragment downstream of *kanR* and oriented so that transcription is in the same direction as the *kan* promoter out across the transposon end. This cassette is inserted into the HindIII site (original nt 2272) of the ATS transposase-containing EcoRI fragment of pNK2881 (ATCC 77351). Transcription from the Ptac promoter is in a direction opposite to that of *amp*. pBR322 was modified by deletion of bp 75-2352 and destruction (by filling in) of the HindIII site. The order of the major features in this plasmid is: EcoRI Ptac ATS transposase mini-Tn10 *kan* Plac-UV5 EcoRI pMB1 ori *ampR*. Restriction digests of the clone gave the following bands (in kb): EcoRI 4.6, 2.0; HindIII 4.6, 1.0, 0.9; PstI 6.4. -----ATCC staff

Designation: pNK2887

Insert Information

DNA: genomic

Insert information:

Insert size (kb): 4.5

Gene : Transposase, Tn10, *ats1 ats2* (ATS)

Source : *Escherichia coli*

DNA : genomic

Insert ends : EcoRI

Insert lengths(kb): 4.5

Gene product: transposase, Tn10, *ats1 ats2* (ATS)

Target Gene: transposase, Tn10, *ats1 ats2* (ATS)

Vector Information

Construct size (kb): 6.599999904632568

Propagation

Aseptically add 0.3 to 0.4 mL of liquid medium to the freeze-dried pellet and mix well. Transfer 100 µL to a test tube containing 5 mL LB+50mg/mL of ampicillin. A loopful of culture can also be streaked on an LB + amp agar plate. Incubate cultures at 37°C. Isolate DNA using standard plasmid preparation procedures.

Growth Conditions

Temperature: 37.0°C

Medium

ATCC® Medium 1227: LB Medium (ATCC medium 1065) with 50 mcg/ml ampicillin

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC




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recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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