





Product Sheet

pRS314 plasmid in *E. coli* (ATCC® 77143™)

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: pRS314 plasmid in *E. coli* (ATCC® 77143™)

Shipping Information

Distributed: freeze-dried

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

One of a series of pBluescript-based centromere vectors (ATCC 77142-77145, 77157-77158) differing in the yeast selectable marker gene. YC-type centromere vector permitting visual detection of recombinants and production of ssDNA in *E. coli*. Contains promoters for in vitro RNA synthesis, priming sites useful for sequencing, and encodes the lacZ alpha (lacZ) peptide. pRSS56, constructed by ligating a PvuI fragment (bp 498-2412) of pBluescript KS+ to a PvuI fragment (bp 2850-730) of pBS(+), contains the KS MCS from pBluescript KS(+) and the unique NdeI and AatII sites between bla and f1 origin of pBS(+). A genomic HindIII/PstI fragment (1.002 kb) containing the TRP1 gene was inserted into the NdeI site and a cassette containing CEN6 and the ARS associated with histone 4 (ARSH4) was inserted into the AatII site of pRSS56. All ends were blunted. An EcoRI site in the TRP1-containing fragment (external to the coding sequence) was destroyed. The order of the major features in this plasmid is: TRP1- f1 ori (NaeI) T7 promoter lacZ/MCS T3 promoter pMB1 ori bla CEN6 ARSH4.

Designation: pRS314 plasmid in *E. coli*

Distribution Host:

Distribution host: *Escherichia coli* HB101 (ATCC 33694)

Propagation

Aseptically add 0.3 to 0.4 mL of liquid medium to the freeze-dried pellet and mix well. Transfer 100 μ L 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

Vector Information

Size (kb): 4.785

DESCRIPTION OF VECTOR:

Intact vector size: 4.785

Type of vector: phagemid

Cloning sites: KpnI Apal XhoI Sall ClaI EcoRI PstI SmaI BamHI SpeI EagI

NotI SacII SacI NaeI

Polylinker sites: KpnI Apal XhoI Sall ClaI HindIII EcoRV EcoRI PstI SmaI BamHI

SpeI XbaI EagI NotI BstXI SacII SacI

Construction: pRSS56 [pBluescript KS+, pBS(+)]

Host range: *Saccharomyces cerevisiae*/Candida robusta; *Escherichia coli*

Features (with orientation and position when available):

marker(s): TRP1, -, 468-1142

replicon: f1, -, 1463-1562

insert detection: lacZ', <-, 1696-2033

promoter for in vitro transcription: T7, ->, 1862-1881

MCS: SacI...KpnI, ->, 1889-1996

promoter for in vitro transcription: T3, <-, 2008-2027

promoter: lac, <-

replicon: pMB1, 2451-2451

marker(s): ampR, <-, 3209-4069

centromere: CEN6, 4211-4327

replicon: ARSH4, 4328-4702

Vector: pRS314 (phagemid)

Promoters: Promoter for in vitro transcription T7

Construction: pRSS56 [pBluescript KS+, pBS(+)]

Marker(s):TRP1,ampR

Construct size (kb): 4.785

Features: insert detection: lacZ'

marker(s): TRP1

marker(s): ampR

promoter: lac

promoter for in vitro transcription: T3

promoter for in vitro transcription: T7

replicon: ARSH4


replicon: f1



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replicon: pMB1
MCS: SacI...KpnI
centromere: CEN6

References

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

Notes

Restriction digests of the clone give the following sizes (kb): EcoRI--4.8;
BamHI--4.8; PvuII--4.2, 0.5.
- ATCC staff

One of a series of pBluescript-based centromere vectors (ATCC 77142-77145,
77157-77158) differing in the yeast selectable marker gene.
- Genetics 122: 19-27, 1989

YC-type centromere vector permitting visual detection of recombinants and
production of ssDNA in *E. coli*. Contains promoters for in vitro RNA synthesis,
priming sites useful for sequencing, and encodes the lacZ alpha (lacZ') peptide.
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to a PvuI fragment (bp 2850-730) of pBS(+), contains the KS MCS from pBluescript
KS+ and the unique NdeI and AatII sites between bla and f1 origin of pBS(+).
- Genetics 122: 19-27, 1989

A genomic HincII/PstI fragment (1.002 kb) containing the TRP1 gene was inserted
into the NdeI site and a cassette containing CEN6 and the ARS associated with
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blunted.
- Genetics 122: 19-27, 1989

An EcoRI site in the TRP1-containing fragment (external to the coding sequence)
was destroyed.
- Genetics 122: 19-27, 1989

The order of the major features in this plasmid is: TRP1 - f1 ori (NaeI) - T7
promoter - lacZ'/MCS - T3 promoter - pMB1 ori - bla - CEN6 - ARSH4.
- Genetics 122: 19-27, 1989

Restriction digests of the vector gave the following sizes (in kb): EcoRI 4.8 ; BamHI 4.8 ; PvuII 4.2,
0.5. -----ATCC staff

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
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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