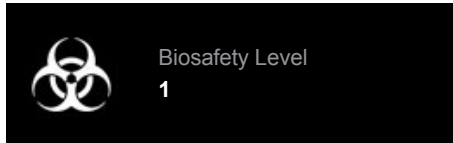




Product Sheet

Escherichia coli integrating vector kit (ATCC® 77371™)

Please read this FIRST



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: (ATCC® 77371™)

Shipping Information

Frozen vials of each as *E. coli* 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 is a set of *Escherichia coli* integrating vectors that differ in their selectable markers (kanamycin, chloramphenicol, tetracycline). Also included is a strain containing a compatible helper plasmid (pLDR8) that provides the lambda *Int* necessary for attP/attB integration.

The components and their ATCC numbers are listed below:

<u>Vector</u>	<u>Marker</u>	<u>ATCC No.</u>
pLDR8	kanR	77357
pLDR9	ampR, kanR	77358
pLDR10	amp, cmlR	77359
pLDR11	ampR, tetR	77360

More specific information on each vector is included below.

Designation: *Escherichia coli* integrating vector kit

Vector Information

ATCC® Number: 77357™

Designation: pLDR8 plasmid in *Escherichia coli*

Description: pLDR8 is compatible with, and provides *int* function for, the vectors pLDR9, pLDR10, and pLDR11. Expression of *int* in pLDR8 is regulated by lambda PR, by the temperature sensitive cI857 repressor. Replication of pLDR8 is also temperature sensitive. Therefore, at 30°C, replication of the plasmid is normal and *int* is not expressed. At 42°C, *int* is expressed but pLDR8 is no longer replicated, resulting in loss of *int* after a few cell generations. The order of the major features in this plasmid are: EcoRI - pSC101 ori - neoR - PstI - cI857 - PR promoter - *int*.

-Plasmid 28: 14-24, 1992

Note: Temperature--resistant derivatives arise frequently. It is important to isolate a clone that is temperature-sensitive and kanamycin-resistant before use.

Vector information:

Vector size (kb): 7.6

Markers: kanR

Distribution host: *Escherichia coli* DH5α

Recommended growth media: LB + kanamycin (25 µg/mL)

Temperature: 30°C (see notes above)

Notes: Restriction digests of the vector gave the following sizes (in kb): EcoRI/PstI - 5.4, 2.2; BamHI - 4.2, 3.4; PstI - 7.6; HindIII - 5.7, 1.9

-ATCC Staff

ATCC® Number: 77358™

Designation: pLDR9 plasmid in *Escherichia coli*

Description: This vector contains the attP sequence for integrating DNA into the lambda attachment site attB. It requires the lambda integrase (*int*) such as that provided by pLDR8. When a recombinant construct is digested with NotI, the origin is separated from the insert. Each fragment has a distinct selectable marker. After religation to form origin-free circles, the products are transformed into bacteria with pLDR8. Expression of *int* in pLDR8 is regulated by lambda PR, by the temperature-sensitive cI857 repressor. Replication of pLDR8 is also temperature sensitive. Therefore, at 30°C, replication of the plasmid is normal and *int* is not expressed. At 42°C, *int* is expressed but pLDR8 is no longer replicated, resulting in loss of *int* after a few cell generations. The order of the major features in this plasmid are: NotI - ampR - ClaI/MCS/EcoRI - attP - NotI - pMB1 ori - kanR.

-Plasmid 28: 14-24, 1992

Vector information:



Product Sheet

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Vector size (kb): 4.1
Markers: ampR, kanR
Replicon: pMB1
Cloning sites: ClaI SalI XbaI BamHI SacI EcoRI
Construction: pT7-7, λ attP, pHSG418 neo

Distribution host: *Escherichia coli* WM1202
Recommended growth media: LB + ampicillin (50 µg/mL)
Temperature: 37°C

Notes: Restriction digests of the vector gave the following sizes (in kb): EcoRI/BglII - 3.7, 0.5; BamHI - 4.2; XbaI - 4.2; NotI - 2.4, 1.8.
-ATCC Staff

ATCC® Number: 77359™

Designation: pLDR10 plasmid in *Escherichia coli*

Description: This vector contains the attP sequence for integrating DNA into the lambda attachment site attB. It requires the lambda integrase (*int*) such as that provided by pLDR8. When a recombinant construct is digested with NotI, the origin is separated from the insert. Each fragment has a distinct selectable marker. After religation to form origin-free circles, the products are transformed into bacteria with pLDR8. Expression of *int* in pLDR8 is regulated by lambda PR, by the temperature-sensitive cI857 repressor. Replication of pLDR8 is also temperature sensitive. Therefore, at 30°C, replication of the plasmid is normal and *int* is not expressed. At 42°C, *int* is expressed but pLDR8 is no longer replicated, resulting in loss of *int* after a few cell generations. The order of the major features in this plasmid are: NotI - ampR - ClaI/MCS/EcoRI - attP - NotI - pMB1 ori - cmlRR.
-Plasmid 28: 14-24, 1992

Vector information:

Vector size (kb): 3.8
Markers: ampR, cmlR
Replicon: pMB1
Cloning sites: ClaI HindIII SalI XbaI BamHI SmaI SacI
Construction: pT7-7, λ attP, pACYC184 cat

Distribution host: *Escherichia coli* WM1202
Recommended growth media: LB + ampicillin (50 µg/mL)
Temperature: 37°C

Notes: Restriction digests of the vector gave the following sizes (in kb): EcoRI/BglII - 1.8, 1.6, 0.5; BamHI - 3.9; XbaI - 3.9; NotI - 2.0, 1.9.
-ATCC Staff

ATCC® Number: 77360™

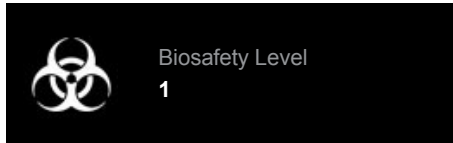
Designation: pLDR11 plasmid in *Escherichia coli*

Description: This vector contains the attP sequence for integrating DNA into the lambda attachment site attB. It requires the lambda integrase (*int*) such as that provided by pLDR8. When a recombinant construct is digested with NotI, the origin is separated from the insert. Each fragment has a distinct selectable marker. After religation to form origin-free circles, the products are transformed into bacteria with pLDR8. Expression of *int* in pLDR8 is regulated by lambda PR, by the temperature-sensitive cI857 repressor. Replication of pLDR8 is also temperature sensitive. Therefore, at 30°C, replication of the plasmid is normal and *int* is not expressed. At 42°C, *int* is expressed but pLDR8 is no longer replicated, resulting in loss of *int* after a few cell generations. The order of the major features in this plasmid are: NotI - ampR - ClaI/MCS/EcoRI - attP - NotI - pMB1 ori - tetR.
-Plasmid 28: 14-24, 1992

Vector information:

Vector size (kb): 4.1
Markers: ampR, tetR
Replicon: pMB1
Cloning sites: ClaI HindIII XbaI SmaI SacI EcoRI
Construction: pT7-7, λ attP, pBR322 tet

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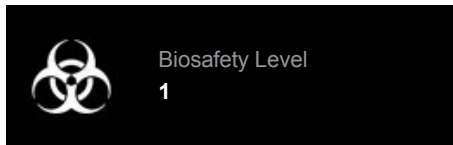
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Shipping Information

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Distribution host: *Escherichia coli* WM1202
Recommended growth media: LB + ampicillin (50 µg/mL)
Temperature: 37°C

Notes: Restriction digests of the vector gave the following sizes (in kb): EcoRI/BglII - 3.6, 0.5; BamHI - 2.2, 1.9; XbaI - 4.1; NotI - 2.3, 1.8.
-ATCC Staff

Propagation

1. Open vial according to instructions.
2. Thaw the vial and incubate cultures at the recommended temperature.
3. Isolate DNA using standard plasmid preparation procedures.

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 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.

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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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