Product Information Sheet for NR-50352

**Escherichia coli – Staphylococcal Shuttle Vectors and Hosts**

**Catalog No. NR-50352**

For research use only. Not for human use.

**Contributor:**
Jeffrey L. Bose, Assistant Professor, Departments of Microbiology, Molecular Genetics and Immunology, University of Kansas Medical Center, Kansas City, Kansas, USA and Eric V. Stabb, Associate Head, Department of Microbiology, Franklin College of Arts and Sciences, University of Georgia, Athens, Georgia, USA

**Manufacturer:**
BEI Resources

**Product Description:**
NR-50352 is a kit containing two vectors that remain stable during both *in vitro* and *in vivo* experiments without the requirement of antibiotics, and two *Escherichia coli* (E. coli) hosts for genetic manipulations prior to transfer into *Staphylococcus aureus* (S. aureus). The shuttle vectors are based on the LAC-p01 plasmid. pKK22 is designed to be used with USA300 strains that contain LAC-p01 and will render the strains isogenic. pKK30 is a variant of pKK22 in which open reading frames (ORFs) not needed for vector maintenance have been deleted and is intended for use in Staphylococcal cells not containing LAC-p01. Both vectors contain a single trimethoprim resistance cassette that is functional in both *E. coli* and *Staphylococcus* species. Additionally, they contain the *E. coli* R6Ky origin of replication, which requires *pir*+ cells for replication. DH5α*Ap*ir and the *pir*+ dam- dcm- strain, GM2163*Ap*ir, are provided as host strains.1-3 The complete nucleotide sequences of pKK22 and pKK30 are available (GenBank: KX085042 and KX085043, respectively) and the vector maps are available below in Appendix I.

**Table 1: *E. coli – Staphylococcus* Vectors and Hosts**

<table>
<thead>
<tr>
<th>BEI Resources Number</th>
<th>Product</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR-50348</td>
<td>pKK22 in <em>E. coli</em> DH5α<em>Ap</em>ir</td>
<td>For use in <em>E. coli</em> DH5α<em>Ap</em>ir or GM2163<em>Ap</em>ir or <em>S. aureus</em> USA300 strains containing LAC-p01²</td>
</tr>
<tr>
<td>NR-50349</td>
<td>pKK30 in <em>E. coli</em> DH5α<em>Ap</em>ir</td>
<td>pKK30 is a variant of pKK22, for use in <em>E. coli</em> DH5α<em>Ap</em>ir or GM2163<em>Ap</em>ir or <em>Staphylococcus</em> species not containing LAC-p01²</td>
</tr>
<tr>
<td>NR-50350</td>
<td><em>E. coli</em> DH5α<em>Ap</em>ir</td>
<td>Host strain containing the <em>pir</em> genes for performing genetic manipulations prior to transfer into <em>Staphylococcus</em> (F<em>Φ80 lacZ ΔM15 ΔlacZYA-argF</em>U169 deoR* supE44 hisD417 recA1 endA1 gyrA96 thi-1 relA1)²</td>
</tr>
</tbody>
</table>

**Material Provided:**
NR-50352 is comprised of 4 vials containing:
- 0.5 mL of vector pKK22, recombinant in *E. coli* DH5α*Ap*ir, in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.
- 0.5 mL of vector pKK30, recombinant in *E. coli* DH5α*Ap*ir, in Tryptic Soy broth containing 10 µg/mL trimethoprim supplemented with 10% glycerol.
- 0.5 mL of *E. coli* DH5α*Ap*ir in Tryptic Soy broth supplemented with 10% glycerol.
- 0.5 mL of *E. coli* GM2163*Ap*ir in Tryptic Soy broth supplemented with 10% glycerol.

**Packaging/Storage:**
NR-50352 was packaged aseptically in cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

**Growth Conditions:**

**Media:**
Tryptic Soy broth, nutrient agar or equivalent
Tryptic Soy agar, nutrient agar, *Staphylococcus* agar with 5% defibrinated sheep blood or equivalent

**Note:** *E. coli* containing pKK22 or pKK30 vectors can be grown with or without 10 µg/mL trimethoprim.

**Incubation:**
- Temperature: 37°C
- Atmosphere: Aerobic

**Propagation:**
1. Keep vial frozen until ready for use, then thaw.
2. Transfer the entire thawed aliquot into a single tube of broth.
3. Use several drops of the suspension to inoculate an agar slant and/or plate.
4. Incubate the tube, slant and/or plate at 37°C for 1 day.

**Citation:**
Acknowledgment for publications should read “The following reagent was contributed by Dr. J. L. Bose for distribution by BEI Resources”.

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BEI Resources
www.beiresources.org

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898

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Biosafety Level: 1

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References:

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APPENDIX I:

Vector pKK22 Map

pKK22 4841 bp

E. coli R6K\text{\textgreek{y}} oriV

Notes:

• pKK22 is designed to be used in USA300 strains of S. aureus containing LAC-p01 (pUSA01)
• Entire plasmid sequence can be found in GenBank Accession KX085042
• \textit{tmpR} denotes trimethoprim resistance in both \textit{E. coli} and \textit{Staphylococcus} species
• Clal site is methylation blocked and sits between the promoter and \textit{dfA} gene
• The R6K\text{\textgreek{y}} origin of replication requires \textit{pir+} strains of \textit{E. coli} to replicate
Notes:

- pKK30 is designed to be used in bacteria not containing LAC-p01 (pUSA01)
- Entire plasmid sequence can be found in GenBank Accession KX085043
- tmpR denotes trimethoprim resistance in both E. coli and Staphylococcus species
- Clal site is methylation blocked and sits between the promoter and dfrA gene
- The R6Kγ origin of replication requires pir+ strains of E. coli to replicate