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SUPPORTING INFECTIOUS DISEASE RESEARCH

Staphylococcus aureus Shuttle Vector pSK5487M, Recombinant in *Escherichia coli*, Strain DH5α

Catalog No. NR-55243

For research use only. Not for use in humans.

Contributor:

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Manufacturer:

BEI Resources

Product Description:

Escherichia coli (\dot{E} . *coli*), strain DH5 α is an ampicillin-resistant cloning host containing the *E. coli*-staphylococcal shuttle vector pSK5487M for use with the *Staphylococcus aureus* (*S. aureus*) cross-resistance panel, available from BEI Resources as NR-55306, for the addition of resistance determinants.^{1,2} Vector pSK5487M contains a 1900 base pair replication region of *S. aureus* multiresistance plasmid pSK41 cloned into the *Eco*Rv site of vector pGEM5Zf, and the chloramphenicol resistance gene (*cat*) cloned between the *sacl* and *sal*I restriction sites for selection in *S. aureus*.^{2,3}

The *S. aureus* cross-resistance panel consists of 22 strains engineered through the introduction of constitutively expressed resistance determinants on plasmid pSK5487M, downstream of the *qac*R promoter, and six spontaneous resistant mutant strains, each with a defined resistance genotype, established in a uniform genetic background of *S. aureus*, strain SH1000. The panel was developed to detect cross-resistance between established and novel antibacterial agents.^{1,2}

Material Provided:

Each vial contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth containing 10 μ g per mL ampicillin supplemented with 10% glycerol.

<u>Note</u>: If homogeneity is required for your intended use, please purify prior to initiating work.

Packaging/Storage:

NR-55243 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:

<u>Media</u>: Tryptic Soy broth or agar containing 10 µg per mL ampicillin or equivalent <u>Incubation</u>: Temperature: 37°C Atmosphere: Aerobic

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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 obtained through BEI Resources, NIAID, NIH: *Staphylococcus aureus* Shuttle Vector pSK5487M, Recombinant in *Escherichia coli*, Strain DH5α, NR-55243."

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. <u>Biosafety in Microbiological and Biomedical Laboratories</u>. 6th ed. Washington, DC: U.S. Government Printing Office, 2020; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

Disclaimers:

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References:

- 1. O'Neill, A. J., Personal Communication.
- Galarion, L. H., et al. "A Platform for Detecting Cross-Resistance in Antibacterial Drug Discovery." <u>J. Antimicrob. Chemother.</u> 76 (2021): 1467-1471. PubMed: 33755133.
- Kwong, S. M., R. A. Skurray and M. Firth. *"Staphylococcus aureus* Multiresistance Plasmid pSK41: Analysis of the Replication Region, Initiator Protein Binding and Antisense RNA Regulation." <u>Mol. Microbiol.</u> 51 (2004): 497-509. PubMed: 14756789.

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