Escherichia coli K-12, Strain IM30B

Catalog No. NR-49807

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Contributor:

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Manufacturer:

BEI Resources

Product Description:

Bacteria Classification: *Enterobacteriaceae, Escherichia*
Species: *Escherichia coli*
Strain: K-12, IM30B
Original Source: *Escherichia coli (E. coli)* K-12, strain IM30B contains the *hsdM* (methylase) and *hsdS* (specificity) genes from *Staphylococcus aureus (S. aureus)* MRSA252 clonal complex 30 (CC).[1,2,4] This insertion mutant was produced in *E. coli* K-12, strain DC10B via recombination-mediated genetic engineering (recombineering).[1,4]
Comments: *E. coli* K-12, strain DC10B is a universal host for constructing plasmids for introduction into staphylococci and was deposited as Δ*dcm*.[2] The lack of methylation allows plasmid DNA to bypass a conserved type IV restriction-modification (RM) barrier in staphylococci which has been identified as a major barrier to transformation with foreign DNA.[1,3,4] *E. coli* K-12, strain IM30B was deposited as resistant to streptomycin.[1,2]

E. coli K-12, strain IM30B is a mutant that allows plasmid DNA to bypass a conserved type IV restriction system (*SauSI*), which was identified as the major barrier to transformation with foreign DNA. Bypassing the *SauSI* restriction barrier allows genetic manipulation of many different staphylococci. Plasmids isolated from strain IM30B transform *S. aureus* at high efficiency and streamline genetic manipulation of major *S. aureus* lineages.[3]

The *S. aureus* CC specific methylation profiles observed in type 1 RM are comprised of three protein components: a methylase (HsdM), a specificity protein (HsdS) and a restriction protein (HsdR). The complex recognizes a target recognition motif (TRM) determined by HsdS and detects its methylation status via HsdM. DNA that is correctly hemimethylated will be fully methylated which will prevent the restriction of the DNA by the RM protein complex.[3] *E. coli* K-12 strains recombineered to contain genes from *S. aureus* CC1, CC8, CC30 and ST93, in a Δ*dcm* background, are available from BEI Resources as NR-49805 through NR-49808, respectively. *E. coli* K-12, strain DC10B (Δ*dcm*) provides a background for plasmid production in the absence of cytosine methylation and is available from BEI Resources as NR-49804.

Material Provided:

Each vial contains approximately 0.5 mL of bacterial culture in Tryptic Soy broth containing 25 µg/mL streptomycin supplemented with 10% glycerol.

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

Packaging/Storage:

NR-49807 was packaged aseptically, in screw-capped plastic 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 or Nutrient broth or equivalent containing 25 µg/mL streptomycin
Tryptic Soy agar or Nutrient agar or Tryptic Soy agar with 5% defibrinated sheep blood or equivalent containing 25 µg/mL streptomycin
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 obtained through BEI Resources, NIAID, NIH: *Escherichia coli* K-12, Strain IM30B, NR-49807."

Biosafety Level: 1

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. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. Monk, I., et al. "Transforming the Untransformable: Application of Direct Transformation to Manipulate Genetically *Staphylococcus aureus* and *Staphylococcus epidermidis*." mBio 20 (2012): e00277. PubMed: 22434850.
2. Monk, I., Personal Communication.
3. Monk, I., et al. "Complete Bypass of Restriction Systems for Major *Staphylococcus aureus* Lineages." mBio 26 (2015): e00308-15. PubMed: 26015493.
4. Monk, I. and T. J. Foster. "Genetic Manipulation of Staphylococci-Breaking Through the Barrier." Front. Cell Infect. Microbiol. 12 (2012): e00049. PubMed: 22919640.

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