LyoComp GT116

Lyophilized competent *E. coli* GT116 cells

Catalog # lyo-116-11, lyo-116-21

For research use only

Version # 09G07-MM

PRODUCT INFORMATION

Content:

LyoComp GT116 cells are provided lyophilized and are available in two sizes;
- lyo-116-11: 4 x 0.5 ml (5 - 10 transformations)
- lyo-116-21: 4 x 1 ml (10-20 transformation)

With each product, 8 ml of sterile reconstitutive solution is provided.

GT116 Genotype: *F- mcrA Δ(mrr-hsdRMS-mcrBC) ϕ80lacZM15 ΔlacX74 recA1 rspL (StrA) endA1 ΔsbcC-sbcD*

Storage and stability:
- LyoComp cells are shipped at room temperature.
- Upon receipt, store LyoComp cells at -20°C.
- LyoComp cells are stable for 6 months when stored at -20°C.

Quality control:

The transformation efficiency of LyoComp cells are evaluated periodically and are guaranteed to be stable for 6 months when properly stored (-20°C).

DESCRIPTION / PROPERTIES

GT116 is a *sbcCD* deletion strain specifically engineered to support the growth of plasmid DNAs carrying hairpin structures, such as psiRNA a vector expressing small interfering RNA. Hairpin structures are known to be unstable in *E. coli* due to their elimination by a protein complex called SbcCD that recognizes and cleaves hairpins[1]. To increase their stability in *E. coli*, InvivoGen has developed GT116 by deleting the *sbcC* and *sbcD* genes. This modification significantly improves the number of recombinant clones harboring a plasmid with hairpin structures. This strain contains the *rpsL* (StrA) gene which confers resistance to streptomycin.

LyoComp GT116 was specifically designed for transformation with psiRNA ligation products or recombinant plasmids. LyoComp GT116 cells exihibit a lower competency than standard chemically competent cells but the competency is sufficient to allow successful cloning into psiRNA plasmids.

Transformation efficiency: 1 x 10^6 cfu/µg

1. Connelly JC. et al., 1998. The SbcCD nuclease of escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. Proc. Natl. Acad. Sci. USA 95:7969-7974

TRANSFORMATION

The following protocol describes a method used to introduce DNA into bacterial host for efficient and convenient construction or maintenance of plasmid recombinants, and blue/white screening.

Additional required materials to be supplied by user:
- LB agar plates with appropriate antibiotic. For optimal results we recommend the use of InvivoGen's selective Fast-Media®.
- 37˚C shaking Incubator
- Ice bucket
- 42˚C water bath
- LB or SOC medium

Method:

Before starting:
- Prepare LB agar plates containing the appropriate antibiotic.
- Set water bath to 42˚C.
- Pre-chill appropriate number of 1.5 ml sterile tubes in ice.

1- Place the appropriate number of LyoComp GT116 cell vials (100 µl per ligation or transformation reaction) in ice for 5 minutes.
2- Rehydrate LyoComp GT116 using cold reconstitutive solution
- for lyo-116-11, add 0.5 ml per vial
- for lyo-116-21, add 1 ml per vial

and store in ice for 5 minutes.
3- Gently homogenize and allow the cells to completely rehydrate in ice for 25-30 minutes.
4- Introduce 10 µl of ligation product (or 1 µg supercoiled plasmid DNA in pre chilled 1.5 ml tubes and return tubes to ice.
5- Gently flick the cells twice to homogenize and add 100 µl of cells to each DNA-containing tubes.
6- Mix by tapping gently and place in ice immediately.
7- Incubate the tubes in ice for 30 minutes.
8- Incubate the tubes in a 42˚C water bath for exactly 30 seconds, then place the tubes back in ice for 1-2 minutes.
9- Add 900 µl of room temperature SOC (or LB) medium to each reaction. (Practice sterile techniques to avoid contamination.)
10- Incubate tubes at 37˚C for 1h30 with shaking at 250 rpm.
11- Spread each transformation reaction (4 x 150 µl if using a ligation product or 100 µl of 10^{-1} and 10^{-2} dilutions if using a supercoiled plasmid) onto LB agar plates containing the appropriate antibiotic.
12- Incubate plates at 37˚C overnight.

TECHNICAL SUPPORT
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