

ChemiComp GT115

Chemically competent *E.coli* GT115 cells

Catalog # gt115-11, gt115-21

For research use only

Version # 09G07-MM

PRODUCT INFORMATION

Content:

ChemiComp GT 115 are provided frozen and are available in two sizes;

- gt115-11: 5 x 0.1 ml (5-10 transformations)
- gt115-21: 5 x 0.2 ml (10-20 transformations)

GT115 Genotype: *F' mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 recA1 rpsL (StrA) endA1 Δdcm uidA(ΔMluI)::pir-116 ΔsbcC-sbcD*

Storage and stability:

- All chemically competent cells are shipped on dry ice.
- Upon receipt, store competent cells immediately at -80°C
- Competent cells are stable for 6 months when properly stored

Quality control:

The transformation efficiency of chemically competent cells are evaluated periodically and are guaranteed to be stable for six months when properly stored.

Special Handling:

Upon receipt, verify that the dry ice is still present in shipping box and that the competent cells are not thawed. Immediately place the competent cells at -80°C. Transformation efficiency may decrease with each freeze and thaw cycle.

DESCRIPTION / PROPERTIES

GT115 is a strain of *E. coli* that has been specifically engineered to support the growth of plasmid DNAs harboring the R6Kγ origin of replication and carrying hairpin structures, such as pCpG-mcs, pCpG-LacZ and pCpG-siRNA vectors. GT115 has the *pir* gene that encodes the π protein which is required by vectors utilizing the R6Kγ ori. Hairpin structures are known to be unstable in *E. coli* due to their elimination by a protein complex called SbcCD that recognizes and cleaves hairpins¹. To increase their stability in *E. coli*, we developed GT115 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.

Transformation efficiency: 0.1-1 x 10⁹ cfu/μg

TRANSFORMATION

Introduction:

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 SOB medium for plating

Method:

Before starting:

- Prepare LB agar plates containing the appropriate antibiotics.
- Set water bath to 42°C.
- Pre-chill appropriate number of 1.5 ml tubes in ice.

- 1- Thaw the appropriate number of competent cells on ice (50μl per ligation or transformation reaction). Allow the cells to thaw on ice for 2-5 minutes.
 - 2- Gently flick the cells twice to resuspend cells. Pipet 50μl of cells to pre chilled 1.5ml tubes and return tubes to ice.
 - 3- Add 1-5 μl of ligation reaction or plasmid DNA to thawed cells. Mix by tapping gently and place on ice immediately.
 - 4- Incubate the tubes on ice for 30 minutes.
 - 5- Incubate the tubes in a 42°C water bath for exactly 30 seconds.
 - 6- Place the tubes back on ice for 1-2 minutes.
 - 7- Add 450 μl of room temperature LB or SOC medium to each reaction. (Practice sterile techniques to avoid contamination.)
 - 8- Incubate tubes at 37°C for 1 hour with shaking at 250 rpm.
 - 9- Spread 50-200 μl of each reaction to separate, labeled LB agar plates containing the appropriate antibiotic.
- Note: For high efficiency transformation rates, 10⁻¹ to 10⁻⁴ dilution of reaction should be spread.*
- 10- Incubate plates at 37°C overnight.

Reference

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

TECHNICAL SUPPORT

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com



3950 Sorrento Valley Blvd. Suite A
San Diego, CA 92121 - USA