

ChemiComp GT115

Chemically competent *E. coli* GT115 cells

Catalog code: gt115-11, gt115-21

<https://www.invivogen.com/chemicomp-gt115>

For research use only

Version 20J14-MM

PRODUCT INFORMATION

Contents

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

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

GT115 Genotype: *F mcrA Δ(mrr-hsdRMS-mcrBC) φ80lacZΔM15 ΔlacX74 nupG recA1 araD139 Δ(ara-leu)7697 galE15 galK16 rpsL(StrA) endA1 Δdcm uidA(ΔMluI)::pir-116 ΔsbcC-sbcD*

Biosafety level: 1

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 from date of receipt when properly stored.

Quality control

The transformation efficiency of chemically competent cells are evaluated periodically and are guaranteed to be stable for six months from date of receipt 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 and pCpG-LacZ 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

1. Connolly JC. et al., 1998. The SbcCD nuclease of escherichia coli is a structural maintenance of chromosomes (SMC) family protein that cleaves hairpin DNA. PNAS 95:7969-74.

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 (not provided)

- LB agar plates with appropriate antibiotic.
- 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 (50 μl 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 50 μl of cells to pre-chilled 1.5 ml 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.

RELATED PRODUCTS

Product	Description	Cat. Code
Blasticidin	Selection antibiotic	ant-bl-1
Hygromycin B Gold	Selection antibiotic	ant-hg-1
Zeocin™	Selection antibiotic	ant-zn-1

TECHNICAL SUPPORT

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