Product Manual

Product name: GlycFree (λDE3) Chemically Competent *E. coli* Cells.
Catalog# GF-201, **Size**: 10 X 0.1 ml/tube

Product details:
The protein expression strain GlycFree (λDE3) is the λDE3 lysogen of E. coli M226 strain (M226((λDE3)). It has been engineered for expression of sugar-free recombinant proteins in *E. coli*. Some glycosyltransferases have ability to catalyze a self-glycosylation reaction using UDP-glucose (UDPG) as glucose donor. As such, these proteins can be aberrantly glycosylated during expression in common *E.coli* strains like BL21 and its derivatives. GlycFree (λDE3) is an *E. coli* strain defective in the synthesis of UDP-glucose. GlycFree(λDE3) allows to produce non-glycosylated forms of target proteins in native conformation and preserve their biological activity.
GlycFree (λDE3) strain carries a copy of the T7 RNA polymerase under control of the IPTG inducible lacUV5 promoter, and as a result, is suitable for controlled expression of both *E. coli* and T7 promoter driven constructs.
ΔUDPG strains are not susceptible to phage P1 infection.
Transformation efficiency: >1 x 10^5 cfu/µg pUC19

E. coli genotype: *e14- galU106 trp-50 relA1 spoT1 rpsL150*(str^R) *(λDE3)*
[λDE3 = λ sBamHIo ΔEcoRI-B int::(lacI::PlacUV5::T7 gene1) i21 Δnin5]

Suggested Transformation Procedure for Optimal Results:

1. Remove cells from -80°C and let thaw on ice.
2. Gently mix cells by lightly flicking tube. Aliquot ~50-100 µl of cells into chilled, 17 x 100 mm polypropylene tube(s). Unused cells may be refrozen, but a drop in efficiency may result. For optimal recovery, refreeze cells in a dry ice/ ethanol bath prior to -80°C storage.
3. Add DNA solution (≤5 µl per 50 µl cells) to cell suspension and gently swirl tube(s) for a few seconds to mix.
4. Incubate on ice for 30 minutes.
5. Place tube(s) in 42°C water bath for ~30 to 45 seconds without shaking. For 50 µl aliquots, 30 seconds is recommended for maximum efficiency.
6. Place tube(s) on ice for ~2 minutes.
7. Add 200-250 µl SOC to transformation reaction(s).
   SOC medium: 2% Tryptone, 0.5% Yeast Extract, 0.4% glucose, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$ & 10 mM $MgSO_4$. Other media can be used to grow transformed cells, including standard LB or TB broths. However, SOC is the optimal choice for recovery of the cells and for obtaining maximum transformation efficiencies.
8. Shake tube(s) ~200 rpm for 1 hour at 37°C.
9. Plate by spreading 100-200 µl of cell transformation mixture on LB agar plates containing appropriate antibiotic and incubate overnight at 37°C.

Notes: If necessary, the entire amount of transformation mixture can be spread on the plates. For that, spin 1 ml of the mixture in microcentrifuge at 10,000 rpm for 30 seconds to pellet cells. Remove 800-900 µl of supernatant. Resuspend cells in the remaining volume of media (100-200 µl) and plate cells (using all volume) onto a pre-warmed selective plate.

Storage is recommended at -80°C.

Usage: This product is intended for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.