

λDE3 Lysogenization Kit

About the Kits

λDE3 Lysogenization Kit	10 rxn	69734-3
λDE3 Lysogenization Kit plus pLysS & pLysE	10 rxn	69725-3

Description

The λDE3 Lysogenization Kit is designed for site-specific integration of λDE3 prophage into an *E. coli* host chromosome, such that the lysogenized host can be used to express target genes cloned in T7 expression vectors. Lysogens are prepared by co-infection with λDE3, a Helper Phage (B10), and a Selection Phage (B482). Lysogens can be verified by plating with the T7 Tester Phage (4107).

λDE3 is a recombinant phage carrying the cloned gene for T7 RNA polymerase under *lacUV5* control. λDE3 (*imm²¹ Anin5 Sam7*) was created by inserting the T7 RNA polymerase gene behind the *lacUV5* promoter using the *Bam*H I cloning site of λD69. Cloning into the *Bam*H I site of λD69 interrupts the *int* gene; therefore, λDE3 cannot integrate into (or be excised from) the chromosome by itself. The Helper Phage provides the *int* function that λDE3 lacks, but cannot form a lysogen by itself because it is *cI*⁻ (has no repressor). The Selection Phage can neither kill λDE3 lysogens, because they have the same immunity, nor integrate into susceptible cells (*cI*⁻), but does kill a major class of λDE3 host range mutants that otherwise would be among the surviving cells. The Tester Phage, a T7 RNA polymerase deletion mutant, is unable to make a plaque on cells that lack T7 RNA polymerase, but makes normal plaques on λDE3 lysogens in the presence of IPTG. The kit also contains a Positive Control lysogen. All phage are provided as clarified lysates, and the Positive Control lysogen is provided as a glycerol stock.

An optional configuration includes plasmids pLysS and pLysE (1 μg each), which can be transformed into the host for additional control over basal expression levels.

Components

- 200 μl λDE3 Phage Lysate
- 200 μl Helper Phage Lysate
- 200 μl Selection Phage Lysate
- 50 μl T7 Tester Phage Lysate
- 200 μl HMS174(DE3) Positive Control Glycerol Stock

Plus pLysS and pLysE kit also contains:

- 1 μg pLysS Plasmid DNA
- 1 μg pLysE Plasmid DNA

Storage

All components should be stored at -70°C.

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Lysogenization

1. Grow host strain in LB supplemented with 0.2% maltose, 10 mM MgSO₄, and any appropriate antibiotics at 37°C to an OD₆₀₀ of 0.5.
2. Store host cells at 4°C until needed (up to 24 h).
3. Using stock lysates, as provided, mix 10⁸ pfu λDE3, 10⁸ pfu Helper Phage, and 10⁸ pfu Selection Phage with 1–10 µl host cells. Use several amounts of host cells within this range to produce plates containing 50–200 candidate lysogens as isolated colonies.

Note: See individual tube labels for phage titers.

4. Incubate host/phage mixture at 37°C for 20 min to allow phage to adsorb to host.
5. Pipet mixture onto an LB plate (supplemented with antibiotics if necessary to select for the host cells). Spread the mixture evenly using a standard spreader or ColiRollers™ Plating Beads (Cat. No. 71013).

Note: To use ColiRollers, dispense 10–20 beads per plate. Cover plate with lid and move plate back and forth several times. The rolling action of beads distributes cells. Several plates can be stacked and processed at one time. After all plates have been spread, discard ColiRollers and incubate.

6. Allow excess moisture to absorb into plates. Invert plates and incubate at 37°C overnight. Most surviving colonies should be λDE3 lysogens.

Verification of λDE3 Lysogens

λDE3 lysogen candidates can be evaluated by their ability to support the growth of the T7 Tester Phage (4107). T7 Tester Phage is a T7 phage deletion mutant that is completely defective unless active T7 RNA polymerase is provided by the host cell. The T7 Tester Phage makes very large plaques on authentic λDE3 lysogens in the presence of IPTG, while much smaller plaques are observed in the absence of inducer. The relative size of the plaques in the absence of IPTG is an indication of the basal level expression of T7 RNA polymerase in the lysogen, and can vary widely between different host cell backgrounds.

The following assay should be performed by plating 100 µl T7 Tester Phage that has been diluted to a titer of 1–2 × 10³ pfu/ml. The supplied phage stock should remain stable for many months at –70°C, but more dilute phage stocks are generally less stable.

Note: T7 phage do not infect male strains of E. coli, therefore, verification of male (F') λDE3 lysogens by this method is not appropriate. The presence of the λDE3 prophage in a male strain is demonstrated by resistance to infection by the Selection Phage, which is of the same immunity group. Alternatively, T7 RNA Polymerase Monoclonal Antibody (Cat. No. 70566) can be used to verify the presence of T7 RNA polymerase in the host strain by Western blot analysis.

1. Grow host strain in LB supplemented with 0.2% maltose, 10 mM MgSO₄, and any appropriate antibiotics at 37°C to an OD₆₀₀ of 0.5.
2. Store host cells at 4°C until needed (up to 24 hr).
3. Dilute an aliquot of T7 Tester Phage in 1X Phage Dilution Buffer to a titer of 1–2 × 10³ pfu/ml.
4. In duplicate tubes, mix 100 µl host cells with 100 µl diluted phage.
5. Incubate host/phage mixture at room temperature for 10 min to allow phage to adsorb to host.
6. Add 3 ml molten top agarose (no warmer than 47°C) to each tube containing host and phage. Pour contents of one duplicate onto an LB plate and the other duplicate onto an LB plate supplemented with 0.4 mM IPTG (isopropyl-β-thiogalactopyranoside) to evaluate induction of T7 RNA polymerase.
7. Allow plates to sit undisturbed for a few minutes until the top agarose hardens. Incubate inverted at 37°C for 2–4 hr, or at room temperature overnight.

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Interpretation of Results

In the absence of IPTG, the observation of very small plaques with discrete edges is an indication that basal level expression of T7 RNA polymerase is low. Cells that have high uninduced levels of T7 RNA polymerase give plaques that are large and have diffuse edges or “halos”. If possible choose lysogens with low basal expression levels for protein production from pET vectors.

In the presence of IPTG, λ DE3 lysogens give very large plaques with big halos around them.

Grow stocks from isolated colonies of the lysogens of choice to an OD₆₀₀ of 0.5–0.8. Add 0.1 vol 80% glycerol. Store at –70°C.

Recipes

Top agarose

per 100 ml:
1 g tryptone
0.5 g NaCl
0.6 agarose
Autoclave

10X Phage Dilution Buffer

per 100 ml of 10X buffer:
20 ml 1 M Tris-HCl, pH 7.4
20 ml 5 M NaCl
10 ml 1 M MgSO₄
Autoclave

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