# One Shot™ Stbl3™ Chemically Competent *E. coli*

**Catalog Number** C7373-03 (20 reactions)

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**WARNING!** This product contains irritants and may be harmful if swallowed. Review the Material Safety Data Sheet before handling. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

## Product description

The Stbl3™ *E. coli* strain is derived from the HB101 *E. coli* strain and is recommended for use when cloning unstable inserts such as lentiviral DNA containing direct repeats (*e.g.* ViraPower™ Lentiviral Expression Kits). The transformation efficiency of Invitrogen™ One Shot™ Stbl3™ chemically competent cells is greater than 1 x $10^8$ cfu/µg DNA.

### Genotype

F$^-$ *mcr*B *mrr hsd*S20(r$_B^-$, m$_B^-$) *rec*A13 *sup*E44 *ara*-14 *gal*K2 *lac*Y1 *pro*A2 *rps*L20(Str$^R$) *xyl*-5 $\lambda^-$ *leu mtl*-1

**Note:** This strain is *end* A1+.

## Contents and storage

| Contents | Quantity | Storage conditions |
|---|---|---|
| Stbl3™ Cells | 21 × 50 µL | Store at –80°C |
| pUC19 Control DNA (10 pg/µL) | 50 µL | |
| S.O.C. Medium | 6 mL | |

## Important guidelines

Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting. Thaw One Shot™ competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by swirling or tapping the tube gently. *Do not mix cells by pipetting*.

**Note:** Cells cannot be used for blue/white screening of plasmid inserts.

## Before you begin

Perform the following before starting the transformation procedure:

1. Equilibrate a water bath to 42°C.
2. Warm the vial of S.O.C. Medium (supplied with the kit) and LB Medium (if needed) to room temperature.
3. Warm the selective plates in a 37°C incubator for 30 minutes (use 1 or 2 plates for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/mL ampicillin.

## Methods

### Transform One Shot™ Stbl3™ chemically competent *E. coli*

We recommend including the pUC19 control plasmid DNA supplied with the kit (10 pg/µL in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8) in your transformation experiment to verify the efficiency of the competent cells. Do not use these cells for electroporation.

1. Thaw, on ice, one vial of One Shot™ Stbl3™ chemically competent cells for each transformation.
2. Add 1 to 5 µL of the DNA (10 pg to 100 ng) into a vial of One Shot™ cells and mix gently. *Do not mix by pipetting up and down.* For the pUC19 control, add 10 pg (1 µL) of DNA into a separate vial of One Shot™ cells and mix gently.
3. Incubate the vial(s) on ice for 30 minutes.
4. Heat-shock the cells for 45 seconds at 42°C without shaking.
5. Remove the vial(s) from the 42°C bath and place them on ice for 2 minutes.
6. Add 250 µL of pre-warmed S.O.C. Medium to each vial.
7. Cap the vial(s) tightly and shake horizontally at 37°C for 1 hour at 225 rpm in a shaking incubator.
8. Spread 25–100 µL from each transformation on a pre-warmed selective plate and incubate overnight at 37°C. We recommend that you plate two different volumes to ensure that at least one plate will have well-spaced colonies. For the pUC19 control, dilute the transformation mix 1:10 into LB Medium (*e.g.* remove 100 µL of the transformation mix and add to 900 µL of LB Medium) and plate 25-100 µL.
9. Store the remaining transformation mix at 4°C. Additional cells may be plated out the next day, if desired.
10. Invert the selective plate(s) and incubate at 37°C overnight.
11. Select colonies and analyze by plasmid isolation, PCR, or sequencing.

### Calculate transformation efficiency

Use the following formula to calculate the transformation efficiency as transformants (in cfu) per µg of plasmid DNA. Remember that the total volume of the transformation mixture is 300 µL.

Transformation efficiency (# transformants/µg DNA) =

$$\frac{\text{\# of colonies}}{\text{10 pg pUC19 DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µL total volume}}{\text{X µL plated}} \times \text{dilution factor}$$

### Example

If transformation of 10 pg of pUC19 DNA yields 40 colonies when 25 µL of a 1:10 dilution is plated, then the transformation efficiency is:

$$\frac{\text{40 colonies}}{\text{10 pg DNA}} \times \frac{10^6 \text{ pg}}{\text{µg}} \times \frac{\text{300 µL total volume}}{\text{25 µL plated}} \times 10 = 4.8 \times 10^8 \text{ cfu/µg}$$

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