Veggie™ Singles™ Competent Cells

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The pET Host Strains (DE3 lysogens) are covered by US Patent 5,693, 489.

For academic and non-profit laboratories, a non-distribution agreement, Technical Bulletin (TB053), can be downloaded from www.merck4biosciences.com. Commercial laboratories must obtain a license agreement from Brookhaven Science Associates prior to purchase of the products.

About the Kits

Veggie™ BL21(DE3) Singles™ Competent Cells	11 rxn	71252-3
	22 rxn	71252-4
Veggie BL21(DE3)pLysS Singles Competent Cells	11 rxn	71253-3
	22 rxn	71253-4
Veggie NovaBlue Singles Competent Cells	11 rxn	71251-3
	22 rxn	71251-4

Description

Veggie Singles Competent Cells are maintained and manufactured with medium and reagents derived from non-animal sources, making these cells ideally suited for applications in which animal-free materials are desired. A Certificate of Origin is included with every shipment. The cells are grown and made chemically competent by an optimized procedure, followed by verification of cloning efficiency and strain identity. The cells are provided as single-use 50 µl aliquots packed in kits for either 11 or 22 transformations. Reproducible high efficiencies are available in three *E. coli* strains, including NovaBlue for routine cloning, blue/white screening (with appropriate plasmids) and plasmid preparation, as well as the pET expression strains BL21(DE3) and BL21(DE3)pLysS for superior performance in T7 promoter-driven protein expression applications.

Components

- 11 x 50 µl or 22 x 50 µl competent cells
- 2 x 2 ml or 4 x 2 ml Veggie SOC Medium
- Test Plasmid (ampicillin resistant)

Storage

Store all components at –70°C or below.

Strain information

Strains offered as competent cells are listed in the table below. Genotypes are listed on page 8.

Strains having the designation (DE3) are lysogenic for the λDE3 prophage that carries an IPTG-inducible T7 RNA polymerase. λDE3 lysogens are used for protein expression from pET, pETcoco™, pETDuet™, and pACYCDuet™ vectors. Strains having the pLysS designation carry a pACYC184-derived plasmid that encodes T7 lysozyme, a natural inhibitor of T7 RNA polymerase, that serves to reduce basal expression of T7 promoter-driven target genes in λDE3 lysogens.

Strain	Resistance[1]	Derivation	Key Feature(s)
BL21(DE3)	none	B834	*lon* and *ompT* protease deficient
BL21(DE3)pLysS	Cam	B834	*lon* and *ompT* protease deficient Reduces basal T7 RNA polymerase activity, facilitates cell lysis
NovaBlue	Tet	K-12	*recA*, *endA*, *lacI*^{q} ; recommended for cloning, plasmid preps

1. The Resistance column in the table refers to selectable marker(s) possessed by the strain in the absence of target plasmids. Appropriate concentrations for selection follows:
   Cam = 34 µg/ml chloramphenicol
   Tet = 12.5 µg/ml tetracycline

BL21 is the most widely used host background for protein expression and has the advantage of being deficient in both *lon* (1) and *ompT* proteases.

NovaBlue is a K-12 strain ideally suited as an initial cloning host due to its high transformation efficiency, blue/white screening capability (with appropriate plasmids) and *recA endA* mutations, which result in high yields of excellent quality plasmid DNA.

Related products	Size	Cat. No.
Carbenicillin	5 g	69101-3
Chloramphenicol	5 g	220551
Kanamycin Sulfate	25 g	420311
Tetracycline Hydrochloride	25 g	58346
100 mM IPTG Solution	10 x 1.5 ml	70527-3
X-Gal Solution, 40 mg/ml in DMSO	3 x 1.0 ml	71077-3

Animal-free media components

Veggie Peptone (Cat. No. 71280-3) and Veggie Yeast Extract (Cat. No. 71279-3) are available separately for making many types of growth and expression media. Both media components are certified animal-free and have been quality tested to ensure proper growth and maintenance of the cells. Veggie Peptone is obtained from papain-digested soymeal and is supplied as a dry powder. Veggie Yeast Extract is supplied in granulated form. The respective component can be used as a direct replacement for animal-derived tryptone and non-certified yeast extract in bacterial growth medium.

Transformation Protocol for Experienced Users

Note: See the following section for a detailed protocol.

1. Thaw the required number of tubes of cells on ice. Mix gently to ensure that the cells are evenly suspended.
2. Add 1 µl DNA solution directly to the cells. Stir gently to mix.
3. Place the tubes on ice for 5 min.
4. Heat the tubes for exactly 30 sec in a 42°C water bath; do not shake.
5. Place on ice for 2 min.
6. Add 250 µl room temperature Veggie™ SOC Medium to each tube.
7. Selection for transformants is accomplished by plating on media containing antibiotic for the plasmid encoded drug resistance. Additional host-specific antibiotics may also be appropriate to insure maintenance of the host selectable marker(s) (see chart on page 2).

   When using NovaBlue, if selecting for ampicillin resistance: *plate 5–50 µl cells directly on selective medium (plus IPTG/X-gal for plasmids that permit blue/white screening).*
   When using NovaBlue, if selecting for kanamycin resistance: *Incubate at 37°C while shaking (250 rpm) for 30 min prior to plating on selective medium.*
   When using strains other than NovaBlue: *incubate at 37°C while shaking at 250 rpm for 60 min prior to plating on selective medium.*

Transformation - Detailed Protocol

Note: When selecting for the expression of β-lactamase, the antibiotic carbenicillin is recommended over ampicillin. Carbenicillin is less sensitive to the drop in the pH of the growth medium that typically accompanies bacterial growth.

DNA in ligation reactions containing high-quality reagents is suitable for direct addition to Novagen competent cells. Inactivation of the ligase is not required prior to transformation. For transformation, 1 µl of the ligation reaction usually yields sufficient numbers of colonies for screening. Up to 5 µl of the ligation reaction containing high quality reagents can be added to Singles Competent Cells without reducing transformation efficiency.

Plasmid DNA isolated using standard miniprep procedures is also usually satisfactory; however, for maximum efficiency, the sample DNA should be free of phenol, ethanol, salts, protein and detergents, and dissolved in TE buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA) or in water. Transformation efficiencies will generally be 10- to 100-fold higher with supercoiled plasmids than with ligation reactions, so it is often necessary to dilute standard plasmid preparations in TE buffer or water prior to transformation. One microliter containing between 1 and 10 ng plasmid DNA is usually sufficient to produce hundreds of colonies. Note that higher concentrations of DNA will yield a higher number of transformants on the plate, but the transformation efficiency of the cells will decrease.

Handling tips

1. Upon receipt, verify that the competent cells are still frozen and that dry ice is still present in the shipping container. Immediately place the competent cells at –70°C or below. For optimal results, do not allow the cells to thaw at any time prior to use.
2. Keep the cells on ice whenever possible.
3. Handle only the very top of the tube and the tube cap to prevent the cells from warming.
4. To mix cells, flick the tube 1–3 times. *NEVER vortex the competent cells*.

Procedure

1. Remove the appropriate number of competent cell tubes from the freezer (include one extra sample for the Test Plasmid positive control, if desired). Immediately place the tubes on ice, so that all but the cap is surrounded by ice. Allow the cells to thaw on ice for ~2–5 minutes.
2. Visually examine the cells to verify that they have thawed and gently flick the cells 1–2 times to evenly resuspend the cells.
3. (Optional) To determine transformation efficiency, add 1 µl (0.2 ng) Test Plasmid to one of the tubes containing cells. Stir gently to mix and return the tube to the ice. Note that the test plasmid is not manufactured with non-animal derived products.
4. Add 1 µl ligation reaction or purified plasmid DNA directly to the cells. Stir gently to mix and return the tube to the ice, making sure that the tube is surrounded by ice except for the cap. Repeat for additional samples.

   Note: Transformation efficiencies can be increased several fold by diluting the ligation reaction 5-fold with TE or water prior to adding the DNA, or by extracting the ligation reaction twice with 1:1 TE-buffered phenol:CIAA (24:1 chloroform:isoamyl alcohol), once with CIAA, precipitating in the presence of NaOAc, and resuspending in TE or water before adding the DNA to the cells. Novagen Pellet Paint® (Cat. No. 69049) or Pellet Paint NF Co-Precipitant (Cat. No. 70748) is recommended for efficient and convenient precipitations.

5. Incubate the tubes on ice for 5 min.
6. Heat the tubes for exactly 30 sec in a 42°C water bath; do not shake.

   Note: This "heat shock" step is most easily accomplished if the tubes are in a rack that exposes the lower half of the tubes. Hold the rack in the water bath so that the lower halves of the tubes are submerged for 30 sec, and then replace the rack on ice.

7. Place the tubes on ice for 2 min.
8. Add 250 µl room temperature Veggie™ SOC Medium to each tube. Keep the tubes on ice until all have received SOC medium. Selection for transformants is accomplished by plating on medium containing antibiotic for the plasmid encoded drug resistance. Additional host-specific antibiotics may also be appropriate to insure maintenance of the host encoded feature(s) (see chart on page 2).

   When using NovaBlue, if selecting for ampicillin resistance: *an outgrowth step is not required before plating on selective medium.*
   When using NovagBlue, if selecting for kanamycin resistance: *shake at 37°C (250 rpm) for 30 min prior to plating on selective medium.*
   When using strains other than NovaBlue: *incubate at 37°C while shaking at 250 rpm for 60 mi n prior to plating on selective medium.*

   Note: The outgrowth incubation is conveniently performed in a shaking incubator using a test tube rack anchored to the shaking platform. Place each transformation tube in an empty 13 mm x 100 mm glass test tube in the rack. The snap-caps on the transformation tubes prevent them from falling to the bottom of the test tubes, and all transformation tubes remain vertical.

   Note: During the outgrowth (or earlier if omitting outgrowth), place the plates at 37°C. If the plates contain a lot of moisture, place them cover-side up and open the cover ~1/3 of the way to allow the plates to dry for 30–45 min. If the plates do not need drying, keep them closed and place them cover-side down in the 37°C incubator for ~ 20 min prior to plating.

9. Refer to "Plating Technique" in the subsequent section for specific instructions. Spread 5–50 µl of each transformation on LB agar plates containing the appropriate antibiotic for the plasmid and host strain (see page 2). When plating less than 25 µl, first pipet a "pool" of SOC onto the plate and then pipet the cells into the SOC. Please see the next section for additional details on plating technique.

   Important: The appropriate amount of transformation mixture to plate varies with the efficiency of both the ligation and the competent cells. As little as 2 µl will yield several hundred transformants under highly efficient conditions (e.g., with NovaBlue cells giving > 4 x 10 8 cfu/µg). For recombinants in NovaBlue, expect 10^5–10^7 transformants/µg plasmid, depending on the particular insert and the ligation efficiency. Transformations with the pETcoco™ plasmid require a plating volume of 50 µl to obtain sufficient colonies. This is because the pETcoco plasmids are large (> 12,000 bp) and therefore transform E. coli less efficiently than smaller plasmids.

***When using the Test Plasmid**, plate no more than 5 µl of the final NovaBlue transformation mix (e.g., 5 µl of NovaBlue cells at 1 x 10^8 efficiency) or plate 10 µl of any strain with a 2 x 10^6 efficiency in a pool of SOC on an LB agar plate containing 50 µg/ml carbenicillin or ampicillin (because the Test Plasmid carries the ampicillin resistance gene, bla).*

***For blue/white screening of recombinants**, also include IPTG and X-gal in the LB agar. These can be pre-spread on the plates and allowed to soak in for about 30 min prior to plating. Use 35 µl of 50 mg/ml X-gal in dimethyl formamide and 20 µl 100 mM IPTG (in water) per 82 mm plate. Alternatively, X-gal and IPTG can be added to the LB agar at a final concentration of 70 µg/ml and 80 µM, respectively, just prior to pouring the plates.*

10. Let the plates sit on the bench for several min to allow excess liquid to be absorbed, and then invert and incubate overnight at 37°C.

Plating techniques

1. Remove the plates from the incubator. If plating less than 25 µl of the transformation, we recommend plating onto a pool of SOC, which facilitates even colony distribution on the plate surface. Using a sterile pipet tip, place 40–60 µl SOC in the center of a plate for a plating cushion.
2. To remove the transformation sample, flick the transformation tube 5–8 times, open the cap and immediately remove the sample volume from the middle of the transformation reaction.
3. Transfer the sample to the plate by dispensing the sample volume into the SOC cushion. After the sample is out of the pipet tip, use the same tip to pipet up the sample volume's worth of SOC from the cushion edge and dispense that SOC back into the cushion. (This effectively rinses out your pipet tip.)

Plating with ColiRollers™ Plating Beads

To use ColiRollers, simply dispense 10–20 beads per plate. Cover the plate with its lid and move the plate back and forth several times. The rolling action of the beads distributes the cells. Several plates can be stacked up and shaken at one time. After all plates have been spread, discard the ColiRollers and incubate (step 4 below).

Note: ColiRollers Plating Beads (Cat. No. 71013) are glass beads that eliminate the use of the spreader and alcohol flame while evenly distributing cells without the possibility of damage.

Plating with a standard spreader

1. Completely immerse the plating spreader (bent glass rod or equivalent) into ethanol and flame to sterilize. After the flame is extinguished, allow the spreader to cool ~10 seconds prior to placing the spreader on the plate. Place the spreader on the LB agar at the outside of the plate (not touching the pool of cells). This further cools the spreader on the LB agar before spreading the cells.
2. *Slowly* turn the plate while supporting the weight of the spreader.

   Important: Do not press down on the spreader – use just enough pressure to spread the cells.
3. Spread until the sample is evenly distributed on the plate. If the plates are fairly dry, the sample and cushion will quickly absorb into the plate. Once the moisture is absorbed, do not continue spreading. If the plates are wet, spread until the sample is evenly distributed. Do not continue to spread until the sample and cushion have absorbed completely into the plate, as overspreading is lethal to the cells. Instead, after spreading briefly, allow the plates to sit upright at room temperature for ~15 minutes prior to placing them in the 37ºC incubator. This will allow excess moisture to absorb into the plates before the plates are inverted and placed in the incubator.
4. Incubate all plates, cover-side down, in the 37ºC incubator for 15–18 hours. To obtain larger colonies, extend the incubation time slightly (1–2 hours), but beware of the potential for development of satellite colonies with extended incubations (usually > 36 h at 37°C; satellites are not commonly observed when using carbenicillin or kanamycin). If performing blue/white screening, placing the plates in a 4ºC refrigerator for a few hours after the colonies have reached the desired size can enhance blue color development.

Troubleshooting

Problems rarely occur if the above protocols are carefully followed. The Test Plasmid is included with all Novagen® competent cells to use as a positive control.

Problem	Possible solution
Experimental DNA produces no colonies or very low number of colonies, but Test Plasmid (included with the kit) yields expected efficiency	a. Experimental DNA contains an inhibitor of ligation. Make sure input DNA is free of contaminants (e.g. excess salts, EDTA, proteins, etc.) that inhibit ligation. Gel purify and/or extract the vector and insert prior to ligation. b. Experimental DNA contains an inhibitor of transformation. Mix Test Plasmid with the ligation and transform on carb or amp plates. If the expected number of colonies is seen, this is not the problem. If colony number is low, dilute the ligation 5-fold in TE buffer or extract, precipitate and resuspend the ligation in TE buffer prior to transformation. c. Vector and/or insert have damaged or otherwise incompatible ends. Recheck cloning strategy, including vector:insert ratio, and use fresh, reliable reagents for DNA preparation. If cloning PCR products, it is likely to be faster to clone them first using a Perfectly Blunt® or **A**ccep**T**or™ Vector Kit. Then, if needed, transfer into another vector using restriction enzymes to excise the fragment. d. Insert is not tolerated in *E. coli*. If possible, check the target sequence for strong *E. coli* promoters or other potentially toxic elements, as well as inverted repeats. Occasionally, certain repeated elements (usually found only in genomic DNA) are not well-maintained in NovaBlue or other multi-purpose *E. coli* strains. These sequences can sometimes be cloned in *recJ*$^-$ strains. Inserts may have a methylation pattern incompatible with the host strain. e. Verify that IPTG was NOT added to the plate when attempting to transform a DE3 lysogen based host strain. IPTG will induce the expression of T7 RNA polymerase in DE3 hosts and any target gene on a T7 promoter based plasmid. This typically results in decreased fitness of the cell and will likely be selected against over time. IPTG induction of DE3 hosts should ONLY be performed after a stable transformant has been isolated. f. Avoid exceeding the recommended volume of input DNA. g. Transformations of pETcoco™ plasmids may require increasing the volume of transformation mixture plated (e.g. 50 µl) to obtain sufficient colonies because the pETcoco plasmid is large (> 12,000 bp) and therefore transforms *E. coli* less efficiently than smaller plasmids.
No colonies or low colony numbers with the Test Plasmid	a. If no colonies are observed, the incorrect selective drug or the correct selective drug may have been used in the plates. Use ampicillin or carbenicillin at 50 µg/ml with the Test Plasmid. b. Incorrect or toxic media components, or plates too old/dry. Recheck media formulations. c. Incorrect incubator temperature. Make sure incubator is set to 37°C. d. Cells were handled incorrectly. Handle the cells very gently at all times. Never vortex or mix vigorously. To resuspend cells, finger-flick or gently pipet up and down without generating bubbles. Make sure the cells are stored at –70°C or below. Thaw on ice and keep on ice except where indicated in the procedure. Gently resuspend the cells before plating if they settle out during outgrowth. Use a very light touch with the spreader when plating or use ColiRollers™ Plating Beads.
Small “satellite” colonies present	a. Plates were left at 37°C too long. β-lactamase is secreted by amp-resistant bacteria and thus can eventually clear a zone of surrounding media from the drug, allowing non-recombinants to grow. In general, colonies are sufficiently large for analysis after 18 h (NovaBlue) or 15 h (other strains). b. Antibiotic stock is degraded, plates are old or drug was added when the media was too hot. Use freshly prepared antibiotic and correct plate preparation. For the *bla* gene, use the ampicillin analog carbenicillin, which appears to be less susceptible to degradation.

Strain Genotypes

Strain	Genotype
BL21(DE3)	F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3)
BL21(DE3)pLysS	F^- *ompT hsdS*$_B$(r_B^- m_B^-) *gal dcm* (DE3) pLysS (Cm^R)
NovaBlue	*endA1 hsdR17*(r_{K12}^- m_{K12}^+) *supE44 thi-1 recA1 gyrA96 relA1 lac* F'[*proA*$^+$*B*$^+$ *lacI*^{q}*ZΔM15*::Tn*10* (Tc^R)]

Genetic Marker Description

Marker	Description	Marker	Description
dcm	No methylation of cytosines in the sequence CCWGG.	*lacZΔM15*	Lacks coding region for amino terminal portion of ß-galactosidase (aa 11-41).
endA1	Endonuclease I activity absent; thought to improve quality of plasmid minipreps.	*lon*	Deficient for an ATP-dependent protease; thought to stabilize some foreign proteins.
F^-	Strain does not contain the F episome.	*ompT*	Lacks an outer membrane protease; improves recovery of intact recombinant proteins.
F'	Strain contains an F plasmid which harbors some bacterial chromosomal DNA.	pLysS	Contains a Cm^R plasmid (pACYC184) that carries the gene for T7 lysozyme.
gal	Unable to utilize galactose. Allow formation of disulfide bonds in *E. coli* cytoplasm	*proAB*	Requires proline for growth on minimal medium.
gyr	Mutation in DNA gyrase. Confers resistance to naladixic acid.	*recA1*	Abolishes homologous recombination.
hsdR	Abolishes restriction but not methylation of certain sequences (r^- m^+).	*supE*	Amber suppressor strain; inserts gln suppressor tRNA for UAG codon.
hsdS	Abolishes both restriction and methylation of DNA at certain sites (r^- m^-).	*thi*	Requires thiamine for growth in minimal medium.
lac	Unable to utilize lactose.	Tn *10*	Contains the Tc^R transposable element, Tn*10*.
lacI^q	Produces a high level of *lac* repressor.		

References

1. Phillips, T.A., VanBogelen, R.A. and Neidhardt, F.C. (1984) *J. Bacteriol.* **159**, 283–287.

Bacterial Strain Non-distribution Agreement

By purchase of the Origami™ 2, Origami™ B, Rosetta™ 2, RosettaBlue™, Rosetta-gami™, Rosetta-Gami™ 2, or Rosetta-Gami™ B host strains and acceptance of the following terms, Novagen grants a limited license to use the Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B host strains for the cloning and expression of genes. The intent of this license is not to limit the research use of these materials, but to protect against unauthorized commercial distribution of the strains by third parties.

1. The Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B host strains or any derivative therefrom is not to be offered for resale or distributed outside your laboratory.
2. Gene clones and libraries in the Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B host strains may be distributed for research purposes only, provided that the recipient acknowledge the foregoing condition.
3. Commercial customers must obtain a research license agreement from Brookhaven Science Associates before purchasing and using DE3 lysogens of host strains Origami 2, Origami B, Rosetta 2, RosettaBlue, Rosetta-Gami 2, or Rosetta-Gami B.

The initial purchaser may refuse to accept the above conditions by returning the kit unopened and the enclosed materials unused. By accepting or using the kit or the enclosed materials, you agree to be bound by the foregoing conditions.