



HT96™ Competent Cells

Table of Contents

About the Kits	2
Description	2
Components	2
Storage	2
Transformation	3
Protocol	3
Plating Techniques	5
Troubleshooting	6
Academic and Non-profit Laboratory Assurance Letter	7

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The T7 expression system host strains (DE3 lysogens) are covered by US Patent No. 4,952,496. Commercial customers must obtain a license agreement from Brookhaven Science Associates before purchase.

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About the Kits

Quarters™ Competent Cells and QuarterPack™ Competent Cell Arrays are also available. Chemically competent cells from a variety of expression strains are predispensed in 20 µl aliquots into 24-well “Quarter” sections of a 96-well plate. Up to four 24-well sections can be placed into the HT96™ Isothermal Block (available separately) to create a flexible array for protein expression optimization

HT96™ NovaBlue Competent Cells	1 plate	71011-3
	4 plates	71011-4
HT96 BL21(DE3) Competent Cells	1 plate	71012-3
	4 plates	71012-4

Description

Novagen HT96 Competent Cells enable convenient, efficient transformation of plasmids for high-throughput cloning and expression applications. The cells are predispensed in 20-µl aliquots in a robust 96-well polypropylene plate. The wells are individually sealed and have raised rims to prevent cross-contamination. The airtight foil seal may be pierced with standard pipet tips, or peeled off for access. Groups of 24 wells (3 rows of 8 wells each) can be cut from the whole plate when using smaller numbers of samples.

Strains available in HT96 format include NovaBlue and BL21(DE3). The NovaBlue strain is recommended for routine cloning, blue/white screening, plasmid preparation, and protein expression using *E. coli* promoter based systems. The BL21(DE3) strain is widely used for T7 promoter-based protein expression from pET vectors. The designation (DE3) indicates that the host is a lysogen of λDE3, and therefore carries a chromosomal copy of the T7 RNA polymerase gene under control of the *lacUV5* promoter.

Strain	Genotype
NovaBlue	<i>endA1 hsdR17(r_{K12}⁻ m_{K12}⁺) supE44 thi-1 recA1 gyrA96 relA1 lac F'[proA⁺B⁺ lacZΔM15::Tn10 (Tc^R)]</i>
BL21(DE3)	<i>F⁻ ompT hsdS_B(r_B⁻ m_B⁻) gal dcm (DE3)</i>

Components

- 1 or 4 plates HT96 Competent Cells
- 1 or 4 × 14 ml SOC Medium
- 1 or 2 × 10 µl Test Plasmid, 0.2 ng/µl (Amp^R)
- 1 or 4 pkg 8 Cap Strips
- 1 or 4 Plate Lids
- 1 or 4 Reagent Reservoirs

Storage

Store HT96 Competent Cells, SOC Medium, and Test Plasmid at -70°C or below. Store Cap Strips, Plate Lids, and Reagent Reservoirs at room temperature.

Products available separately	Size	Cat. No.
Carbenicillin	5 g	69101-3
Chloramphenicol	5 g	220551
Kanamycin Sulfate	25 g	420311
Streptomycin Sulfate, <i>Streptomyces</i> sp.	100 g	5711
Spectinomycin, Dihydrochloride, Pentahydrate, <i>Streptomyces</i> sp.	10 g	567570
Tetracycline Hydrochloride	25 g	58346
100 mM IPTG Solution	10 × 1.5 ml	70527-3
HT96 Isothermal Block	1	71195-3
ColiRollers™ Plating Beads	1 pkg	71013-3
	5 pkg	71013-4
X-Gal Solution, 40 mg/ml in DMSO	3 × 1.0 ml	71077-3

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Transformation

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. 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, 1 mM EDTA, pH 8.0) 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 1–10 ng plasmid DNA is usually sufficient to produce hundreds of colonies. For cotransformations into expression strains using two supercoiled plasmids, add 1 µl containing 10–40 ng of each plasmid into expression strain competent cells. No more than 1 µl DNA or ligation reaction should be used per 20 µl competent cells to insure optimal transformation efficiency.

Before starting, be sure that equipment and kit components are ready for use. The Novagen HT96™ Isothermal Block (Cat. No. 71195-3) provides efficient thermal transfer for heat shock and cooling.

The HT96 Competent Cells plates can be subdivided into 24-well (3 × 8) sections immediately after removal from the –70°C freezer (do not allow the cells to thaw). Bend the plate at the scored junctions (visible from underside of plate) to break the plastic plate. Cut the foil seal between the sections. Immediately return the unused sections to the –70°C freezer.

- Preheat an HT96 Isothermal Block to 42°C, or set a 96 well thermal cycler to hold temperature at 42°C. An intact HT96 plate may be floated in a 42°C water bath, but separated sections will tip. In either case, exercise caution to avoid contamination.
- A second Isothermal Block can also be cooled on ice, if desired.
- Thaw the required amount of SOC, and equilibrate to room temperature.

Protocol

1. Remove HT96 Competent Cell plate from –70°C freezer. Subdivide into sections, if applicable. Immediately place in ice-cold HT96 Isothermal Block, or directly on ice.
Optional: if desired, ink on foil seal of plate can be removed with a small amount of 100% ethanol.
2. Prior to adding DNA solution, verify the cells have thawed. Gently tap plate once or twice to resuspend cells. Return plate to ice or HT96 Isothermal Block.
Optional: to determine transformation efficiency, add 1 µl (0.2 ng) Test Plasmid to one of the wells.
3. Use one of the following methods to add DNA to wells. In either case, be sure to pipet the DNA directly into the cells, not on the wall of well.
 - a. Remove foil seal entirely, add 1 µl DNA solution to each well, and stir gently with pipet tip. Cover wells with either the 8-Cap Strips or Plate Lid.
 - b. Pierce foil seal with pipet tip, add 1 µl of DNA solution and stir gently to mix. Cover with Plate Lid.
4. Incubate plate in ice-cold HT96 Isothermal Block, or directly on ice for 5 min.
5. Heat shock cells by placing plate at 42°C (preheated HT96 Isothermal Block or thermal cycler) and incubate for exactly 30 s.
6. Return plate to ice-cold HT96 Isothermal Block or place directly on ice and incubate for 2 min.
7. Add 80 µl SOC (room temperature) to each well. Keep plate in ice-cold HT96 Isothermal Block, or on ice, until all wells have received SOC.

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8. **If using NovaBlue and selecting for ampicillin resistance:** plate 5–50 μ l of the transformation reaction directly on LB plates containing 50 μ g/ml carbenicillin or ampicillin plus 12.5 μ g/ml tetracycline. Include IPTG and X-gal for plasmids that permit blue/white screening.

If using BL21(DE3) with any antibiotic or NovaBlue with antibiotics other than ampicillin/carbenicillin: cap or cover plate as appropriate and incubate at 37°C for 60 min (plates can be shaken at 125 RPM for the incubation, but it is not necessary). After outgrowth, resuspend cells by mixing with pipet tip prior to removing an aliquot for plating.

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 approximately 1/3 of the way to allow the plates to dry 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 approximately 20 min prior to plating.

9. Refer to plating technique in subsequent section for specific instructions. Spread 5–50 μ l of each transformation on LB agar plates containing the appropriate antibiotic(s) for the plasmid. Include 12.5 μ g/ml tetracycline for NovaBlue. When plating less than 25 μ l, 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. For recombinants in NovaBlue, expect 10^5 – 10^7 transformants/ μ g plasmid, depending on the particular insert and the ligation efficiency. Transformations with the pETcoco™ plasmid require a plating volume of 50 μ l to obtain sufficient colonies because the pETcoco plasmid is large (21,272 bp).

When using the Test Plasmid, plate no more than 5 μ l of the final transformation mix or plate 10 μ l BL21(DE3) in a pool of SOC on an LB agar plate containing 50 μ g/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 μ l 50 mg/ml X-gal in dimethyl formamide and 20 μ l 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 μ g/ml and 80 μ M, respectively, just prior to pouring the plates.

10. Set plates on bench for several minutes to allow excess liquid to be absorbed. Invert and incubate overnight at 37°C.

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Plating techniques

This section provides additional details on plating the transformed cells to obtain high, reproducible plating efficiencies.

1. Remove plates from the incubator. If plating less than 25 μ l of the transformation, plating onto a pool of SOC facilitates even colony distribution on the plate surface. Using a sterile pipet, dispense 40–60 μ l SOC in center of plate for a plating cushion.
2. Ensure the cells are evenly suspended before removing sample for plating; this can be accomplished by gently pipetting cells up and down.
3. Transfer sample to the plate by dispensing sample volume into SOC cushion. After sample is expelled, rinse out pipet tip by pipetting up a sample volume's worth of SOC from the cushion edge and dispensing SOC back into cushion.

Plating with ColiRollers™ Plating Beads

To use ColiRollers, simply dispense 10–20 beads per plate. Cover the plate with its lid and rock 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 plates have been spread, discard the ColiRollers and incubate (Step 4 below).

Plating with a standard spreader

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

Important: *Do not press down on the spreader- use just enough contact to spread the cells*

3. Spread until sample is evenly distributed on plate. If plates are fairly dry, sample and cushion will quickly absorb into plate. If plates are wet, spread until sample is evenly distributed. Do not continue to spread until sample and cushion have absorbed completely into plate, as overspreading is lethal to the cells. Instead, after spreading briefly, allow plates to sit upright at room temperature for approximately 15 min prior to placing them in 37°C incubator. This will allow excess moisture to absorb into plates.
4. Incubate all plates, cover-side down, in the 37°C incubator for 15–18 h. To obtain larger colonies, extend the incubation time slightly (1–2 h), but be aware 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, place plates at 4°C for a few hours after the colonies have reached the desired size to enhance color development.

ColiRollers Plating Beads (Cat. No. 71013-3) are glass beads that eliminate the use of the spreader and alcohol flame. ColiRollers allow uniform distribution without damage to the cells.

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Troubleshooting

Problems rarely occur if the above protocols are carefully followed. The Test Plasmid is included with all Novagen competent cells for 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	<ol style="list-style-type: none"> Experimental DNA contains an inhibitor of ligation. Ensure input DNA is free of contaminants (e.g. excess salts, EDTA, proteins, etc.) that inhibit ligation. Gel purify and/or extract vector and insert prior to ligation. Transform 1 ng of your uncut plasmid as a positive control. Experimental DNA contains an inhibitor of transformation. Mix Test Plasmid with the ligation and transform. 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. Vector and/or insert have damaged, or otherwise incompatible ends. Reevaluate cloning strategy, including vector:insert ratio, and use fresh, reliable reagents for DNA preparation. If cloning PCR products, it is generally faster to blunt-end clone first, using a Perfectly Blunt[®] or AccepTor[™] Vector Kit. Then, if needed, transfer into another vector using restriction enzymes to excise the fragment. Insert is not tolerated in <i>E. coli</i>. If possible, check the target sequence for strong <i>E. coli</i> 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 <i>E. coli</i> strains. These sequences can sometimes be cloned in <i>recJ</i>⁻ strains. Inserts may have a methylation pattern incompatible with the host strains. 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. Avoid exceeding the recommended volume of input DNA. DNA volumes greater than 1 µl per 20 µl of competent cells will lead to reduced transformation efficiencies. Transformants of pETcoco[™] constructs typically require plating 50 µl of the transformation mixture to obtain sufficient colonies because the vectors are large (>12 kbp), and they transform with a lower efficiency.
No colonies or low colony numbers with the Test Plasmid	<ol style="list-style-type: none"> If no colonies are observed, the incorrect selective drug or the wrong concentration of the correct selective drug may have been used in the plates. Use ampicillin or carbenicillin at 50 µg/ml with the Test Plasmid. Incorrect or toxic media components, or plates too old/dry. Recheck media formulations. Incorrect incubator temperature. Make sure incubator is set to 37°C. 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	<ol style="list-style-type: none"> 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). Antibiotic stock is degraded, plates are old, or drug was added when the media was too hot. Use freshly prepared antibiotics and correct plate preparation. For the <i>bla</i> gene, use the ampicillin analog, carbenicillin, which appears to be less susceptible to degradation.

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The T7 expression system is based on technology developed at Brookhaven National Laboratory under contract with the U.S. Department of Energy and is the subject of patent applications assigned to Brookhaven Science Associates (BSA). This technology, including bacteria, phages, and plasmids that carry the gene for T7 RNA polymerase, is made available on the following conditions:

1. The T7 expression system is to be used for noncommercial research purposes only. A license is required for any commercial use, including use of the T7 system for research purposes or for production purposes by any commercial entity. Information about commercial licenses may be obtained from the Patent Office, Brookhaven National Laboratory, Upton, New York, 11973, Telephone: (631) 344-7134. Contact: Christine Brakel.
2. No materials that contain the cloned gene for T7 RNA polymerase may be distributed further to third parties outside of your laboratory, unless the recipient receives a copy of this assurance letter and agrees to be bound by its terms. This limitation applies to any of the following materials that are included in this kit and to any derivatives you may make of them:

<i>E. coli</i> B834(DE3)	<i>E. coli</i> Origami 2(DE3)	<i>E. coli</i> Rosetta-gami™(DE3)
<i>E. coli</i> B834(DE3)pLysS	<i>E. coli</i> Origami 2(DE3)pLysS	<i>E. coli</i> Rosetta-gami(DE3)pLysS
<i>E. coli</i> BL21(DE3)	<i>E. coli</i> Origami 2(DE3)pLacI	<i>E. coli</i> Rosetta-gami(DE3)pLacI
<i>E. coli</i> BL21(DE3)pLysS	<i>E. coli</i> Origami B(DE3)	<i>E. coli</i> Rosetta-gami 2(DE3)
<i>E. coli</i> BL21(DE3)pLysE	<i>E. coli</i> Origami B(DE3)pLysS	<i>E. coli</i> Rosetta-gami 2(DE3)pLysS
<i>E. coli</i> BL26(DE3)pLysE	<i>E. coli</i> Origami B(DE3)pLacI	<i>E. coli</i> Rosetta-gami 2(DE3)pLacI
<i>E. coli</i> BLR(DE3)	<i>E. coli</i> Rosetta™(DE3)	<i>E. coli</i> Rosetta-gami B(DE3)
<i>E. coli</i> BLR(DE3)pLysS	<i>E. coli</i> Rosetta(DE3)pLysS	<i>E. coli</i> Rosetta-gami B(DE3)pLysS
<i>E. coli</i> HMS174(DE3)	<i>E. coli</i> Rosetta(DE3)pLacI	<i>E. coli</i> Rosetta-gami B(DE3)pLacI
<i>E. coli</i> HMS174(DE3)pLysS	<i>E. coli</i> Rosetta 2(DE3)	<i>E. coli</i> Tuner™(DE3)
<i>E. coli</i> HMS174(DE3)pLysE	<i>E. coli</i> Rosetta 2(DE3)pLysS	<i>E. coli</i> Tuner(DE3)pLysS
<i>E. coli</i> NovaBlue(DE3)	<i>E. coli</i> Rosetta 2(DE3)pLacI	<i>E. coli</i> Tuner(DE3)pLacI
<i>E. coli</i> Origami™(DE3)	<i>E. coli</i> RosettaBlue™(DE3)	Bacteriophage λCE6
<i>E. coli</i> Origami(DE3)pLysS	<i>E. coli</i> RosettaBlue(DE3)pLysS	Bacteriophage λDE3
<i>E. coli</i> Origami(DE3)pLacI	<i>E. coli</i> RosettaBlue(DE3)pLacI	

3. 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.

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