

DG1 Competent cells

**Electro- or chemically- competent *E. coli* cells
supplied in 96-tube tray**

Instruction manual (v1.4)

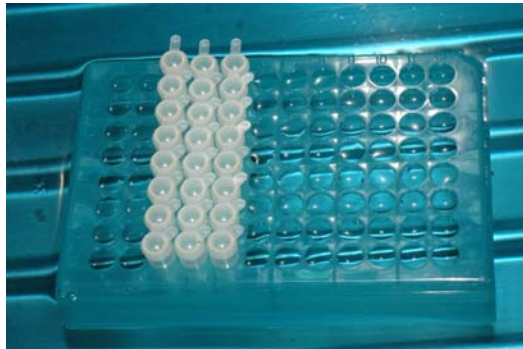


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Content and storage :

The DG1 *E. coli* competent cells are shipped on **dry ice**.

Storage: -70°C to -80°C. The SOC medium can be stored at room temperature.

Two different types of competent cells are available: one containing electrocompetent cells, and the other type containing chemically-competent cells. Electroporation is more efficient than chemical transformation.

Each box contains the following items:

Reference	Content	Amount
GE-DG1C-24	24 uncolored tubes of chemically-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	24 x 50 µl
GE-DG1C-48	48 uncolored tubes of chemically-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	48 x 50 µl
GE-DG1C-96	96 uncolored tubes of chemically-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	96 x 50 µl
GE-DG1E-24	24 green tubes of electro-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	24 x 50 µl
GE-DG1E-48	48 green tubes of electro-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	48 x 50 µl
GE-DG1E-96	96 green tubes of electro-competent DG1 <i>E. coli</i> cells + 1 tube of SOC medium (30ml)	96 x 50 µl
GE-DG1R-00	Empty rack for tubes	

Genotype:

DG1: *mcrA* $\Delta(mrr-hsdRMS-mcrBC, modification-, restriction-)$ $\Phi80lacZ\Delta M15$ $\Delta lacX74$
recA1 araD139 $\Delta(ara-leu)7697$ *galU galK rpsL endA1 nupG*

Material Safety Data Sheet:

Producing Company identification:

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B-6041 Charleroi, Belgium

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e-mail: delphigenetics@delphigenetics.com

Hazards identification

There is no specific hazard concerning the products of the DG1 competent cells.

First aid measures

- Inhalation: If one of the products of the DG1 competent cells is inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
- Ingestion: Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of the products of the DG1 competent cells are swallowed, call a physician immediately.
- Skin contact: In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
- Eye contact: In case of contact with one of the products of the DG1 competent cells, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.

Fire-fighting measures

Use foam or all purpose dry chemicals to extinguish. Fire fighters should wear positive self-contained breathing apparatus and full turnout gear.

Accidental release measures

Immediately contact emergency personnel. Use suitable protective equipment (see below exposure controls and personal protection). For small spills add absorbent, scoop up material and place in a sealed, liquid-proof container for disposal. For large spills dike spilled material or otherwise contain material to ensure runoff does not reach a waterway. Place spilled material in an appropriate container for disposal. Minimize contact of spilled material with soils to prevent runoff to surface waterways.

Handling and storing

Keep the container tightly closed, in a cool and well-ventilated area.

Personal protection

The occupational exposure limits were not determined. Protect your skin and body using uniform or laboratory coat, chemical resistant, impervious gloves. Use safety glasses, face shield or other full-face protection if potential exists for direct exposure to aerosols or splashes.

Disposal consideration

Waste must be disposed of in accordance with federal, state and local environmental control regulations.

N.B.: Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. To the best of our knowledge, the information contained herein is accurate. However, neither Delphi Genetics SA nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein.

User Guide

Introduction:

Competent DG1 *E. coli* cells are supplied in a 96-tube tray. This convenient format gives you the freedom to perform from 1 to 96 transformations at a time, without thawing more tubes of cells than needed. You can remove individual tubes by cutting cap strips. Thus, this format eliminates the reduced efficiencies that occur as a result of repeated freezing and thawing and makes possible high-throughput transformation. Racks feature 8 x 12 holes pattern for rapid processing using multi-channel pipettors. Alpha-numeric, non-reversing, rack and lid ensure positive sample ID. You can use the alpha-numeric grid at page 10 of this manual to identify your tubes.

Each tube contains 50µl of competent *E. coli*, which is sufficient for one high efficiency transformation. To maintain high competency, the bacteria must be stored at -70°C to -80°C.

The DG1 strain is convenient for use in cloning, plasmid preparation and library construction. It is endonuclease and recombination minus. It allows lacZalpha complementation for blue/white screening.

Additional material required:

- Ice bucket with crushed ice
- 42°C water bath (for chemically competent cells only)
- Electroporation cuvettes and apparatus (electrocompetent cells only)
- LB agar plates containing appropriate additives (antibiotics, IPTG, X-gal,...)
- Incubators (37°C) and shakers (37°C).

Protocol:**a) Transformation by electroporation:**

1. Prepare LB plates containing the appropriate additives (antibiotics, IPTG, X-Gal,...). Let the plates dry and then warm them up at 37°C.
2. Set up your electroporator for bacterial transformation. Use the manufacturer's instructions. Classically, electroporation conditions are: 2,5 kV, 25 µF, and 200 Ohms.
3. Thaw (bring to room temperature) the tube of SOC medium.
4. For each reaction, place one tube of the DG1 electrocompetent cells (**green** tube) and one electroporation cuvette on ice. Allow the cells to thaw on ice for 5-10 minutes.

The box that holds the tubes can be used as a rack during the procedure. It is possible to remove the bottom panel of the box to thaw the cells on ice. If you need an additional empty rack, it is possible to receive it with your order. Please add the following reference in your order form: GE-DG1R-00 (empty rack).

5. Add 1 or 2 µl of DNA (ligation product, plasmid preparation) to one tube of the DG1 electrocompetent cells. Stir gently to mix. Do not mix by pipetting up and down.

If you wish to use more than 2µl of DNA, it is recommended to dialyze it against sterile water using a filter with a 0.025µm pore size. Add the sterile water in a Petri dish and carefully place the filter on the water surface. Delicately, put the ligation mix on the filter. Wait 10min, pipet back the ligation mix and add the dialyzed solution to the electrocompetent cells.

Alpha-numeric, non-reversing, rack and lid ensure positive sample ID. You can use the alpha-numeric grid at page 10 of this manual to identify your tubes.

6. Transfer all the content of the tube (cells+DNA) to the pre-chilled electroporation cuvette.
7. Electroporate the cells according to the manufacturer's instructions.

If you experience electric arcing during electroporation, try again with a dialyzed DNA solution or reduce the voltage (10 to 20% less, 2kV instead of 2.5kV for example).
8. Quickly add 1ml of the SOC medium at room temperature and mix well.
9. Incubate tubes at 37°C for one hour with shaking (200 to 250 rpm).
10. Spread 10, 20 and 100 µl of the product (of step 9) on the pre-warmed plates.
11. Incubate the plates overnight at 37°C.

b) Transformation using chemically competent cells:

1. Prepare LB plates containing the appropriate additives (antibiotics, IPTG, X-Gal,...). Let the plates dry and then warm them up at 37°C.

2. Set a water bath or a heating-bloc to 42°C

3. Thaw (bring to room temperature) the tube of SOC medium.

4. For each reaction, place one tube of the DG1 chemically-competent cells (uncolored tube) on ice. Allow the cells to thaw on ice for 5-10 minutes.

The box that holds the tubes can be used as a rack during the procedure. It is possible to remove the bottom panel of the box to thaw the cells on ice or to immerse the tubes in a water bath. If you need an additional empty rack, it is possible to receive it with your order. Please add the following reference in your order form: GE-DG1R-00 (empty rack).

5. Add 1 to 5 µl of the DNA solution (ligation reaction, plasmid extraction,...) to one tube of the DG1 chemically competent cells (uncolored tube). Stir gently to mix. Do not mix by pipetting up and down.

Alpha-numeric, non-reversing, rack and lid ensure positive sample ID. You can use the alpha-numeric grid at page 10 of this manual to identify your tubes.

6. Incubate on ice for 30 minutes.

7. Heat-shock the bacteria by placing the vial at 42°C for 30 seconds without shaking.

It is possible to remove the bottom panel of an empty box to immerse the tubes in the water bath.

8. Immediately transfer the tubes to ice.

9. Add 250µl of room-temperature SOC medium and mix well.

10. Incubate tubes at 37°C for one hour with shaking (200 to 250 rpm)

11. Spread 10, 20 and 100µl of the product (from step 10) on different pre-warmed plates.

12. Incubate the plates overnight at 37°C.

Media information:

LB (per liter):

10g Tryptone

10g NaCl

5g Yeast extract

Dissolve in 1 liter deionized water

Add 15g of agar if you want to prepare LB plates

Autoclave (20min, 121°C) and let cool to ~55°C before adding the appropriate additives (antibiotics, IPTG, X-gal,...).

SOC (per liter):

10g Tryptone

0.5g NaCl

5g Yeast extract

Dissolve in 960ml deionized water

Add 10ml of 250mM KCl stock solution

Autoclave (20min, 121°C) and let cool to ~55°C

Aseptically add 10ml of 1M MgCl₂ stock solution

Aseptically add 20ml of 1M glucose stock solution

Antibiotic final concentration:

Ampicillin: 100 to 500µg/ml

Chloramphenicol: 12.5 to 20µg/ml

Kanamycin: 50 to 100µg/ml

Spectomycin: 100µg/ml

Streptomycin: 100µg/ml

Troubleshooting:

Please note that problems with transformation efficiency can result from the following parameters. Most of these problems can be fixed as explained in the table below. However, due to intrinsic and specific properties of your DNA, the number of recovered recombinants may vary.

Problem	Solution
Arcing during the electroporation step	Check your electroporation conditions. Classical conditions for bacterial electroporation are: 25µF, 2.5kV, 200 Ohms. Electroporate only 1 or 2 µl of your DNA preparation (ligation mix, plasmid preparation). If you want to use more DNA or if you still experience arcing, dialyze your DNA sample using a 0.025µm filter and sterile water.
Only a few or no colonies are observed after transformation	<ul style="list-style-type: none"> √ When using electrocompetent bacteria, check the electroporation conditions (see above). When using chemically competent bacteria: check the temperature of the water bath, incubate the transformation product during one hour at 37°C to allow regeneration of the bacteria before spreading. √ When transforming a ligation mix, check the DNA concentration of your insert. Be sure to use the adequate DNA quantity. Check the quality of your insert using agarose gel electrophoresis. √ Check your plates with another strain which is resistant to the same antibiotic. If no growth is observed, check your antibiotic solution. √ Transform a control plasmid carrying the same antibiotic resistance (use a fresh plasmid DNA extraction). <p><i>Remark: You can check the cloning and electroporation efficiencies by transforming a fresh plasmid DNA (use maximum 10ng of DNA).</i></p>
Several colonies are observed but they are not containing the transformed plasmid	√ Check the SOC medium. Spread 100µl of SOC medium on the plates containing the same antibiotics and incubate at 37°C overnight. If colonies appear, the SOC medium is contaminated. Use fresh SOC medium.

Related Staby™ products and services:



The **StabyCloning™ kit** is designed for the rapid, precise and efficient DNA cloning of PCR products. The complete cloning procedure is performed in one hour (including plating), the background is basically nil (the bacteria containing vectors without insert are killed), the PCR product is oriented, the plasmid is stabilized, and the export of the insert to another vector is easily selected.



The **StabyExpress™ T7 kit** contains all the key elements for cloning of a gene-of-interest and its expression in *Escherichia coli*. The kit combines two technologies (T7 expression and plasmid stabilization) that allow high-yield protein expression and standardization of the production-protocol.



The **GetStaby™ kit** allows easy addition of Delphi-Genetics' stabilization technology into your favourite vector. The technology is compatible with any expression system. Using this technology, your vectors are perfectly stabilized even without antibiotics.



The **Staby™ Codon T7 kit** combines three technologies to ensure high-yield and standardized expression of eukaryote proteins in *Escherichia coli*. These technologies are (i) T7-controlled expression, (ii) plasmid stabilization, and (iii) codon-usage adaptation of *E. coli* for the efficient expression of proteins that contain rare codons.



The **Cherry™ Express kit** allows direct visualization (by eye!) of your protein of interest during protein production in *E. coli* and protein purification. Special requirements or reagents are not needed. It is also possible to quantify the protein concentration at any step by spectral measurement. The Cherry™ Express kit combines multiple advantages: protein visualization, T7 expression, plasmid stabilization and codon-usage adaptation.



The **Staby™ Switch** medium is an auto-inducible medium (ready-to-use) designed for high-level protein expression using Staby™ products or any other IPTG-inducible bacterial expression system. Using Staby™ Switch medium, protein expression is automatically induced when high cell density is reached. Thus, it is neither necessary to add IPTG nor to monitor optical density during bacterial growth.



Staby™ Soft was specifically designed by Delphi Genetics to support the users of the Staby™ Operating System. This software package can perform customized gene-of-interest analysis to choose the most adapted kit and to optimize protein production.

For more information, please, consult www.delphigenetics.com

Staby™ products ordering information:

StabyExpress™		
GE-SET7-0505	StabyExpress T7 expression kit, electro-competent cells	5 reactions
GE-SET7-0707	StabyExpress T7 expression kit, chemically-competent cells	5 reactions
GE-SET7-1010	StabyExpress T7 expression kit, electro-competent cells	10 reactions
GE-SET7-1212	StabyExpress T7 expression kit, chemically-competent cells	10 reactions
GE-SET7-1111	Set of 10 cloning bacteria (CYS21) and 10 expression bacteria (SE1), electro-competent cells	10 reactions
GE-SET7-1313	Set of 10 cloning bacteria (CYS21) and 10 expression bacteria (SE1), chemically-competent cells	10 reactions
GE-SET7-2020	StabyExpress T7 expression kit, electro-competent cells	20 reactions
GE-SET7-2222	StabyExpress T7 expression kit, chemically-competent cells	20 reactions
GE-SET7-0020	Set of 20 expression bacteria (SE1), electro-competent cells, 50µl/tube	20 reactions
GE-SET7-0022	Set of 20 expression bacteria (SE1), chemically-competent cells, 100µl/tube	20 reactions
GetStaby™		
GE-GSA1-10	GetStaby kit, electro-competent cells	10 reactions
GE-GSA1-12	GetStaby kit, chemically-competent cells	10 reactions
StabyCloning™		
GE-STC1-10	StabyCloning kit, electro-competent cells	10 reactions
GE-STC1-12	StabyCloning kit, chemically-competent cells	10 reactions
GE-STC1-20	StabyCloning kit, electro-competent cells	20 reactions
GE-STC1-22	StabyCloning kit, chemically-competent cells	20 reactions
GE-STCB-20	Set of 20 cloning bacteria (CYS21) electro-competent cells (50µl/tube)	20 reactions
GE-STCB-22	Set of 20 cloning bacteria (CYS21) chemically-competent cells (100µl/tube)	20 reactions
Staby™Codon		
GE-SCT7-0505	StabyCodon T7 expression kit, electro-competent cells	5 reactions
GE-SCT7-0707	StabyCodon T7 expression kit, chimio-competent cells	5 reactions
GE-SCT7-1010	StabyCodon T7 expression kit, electro-competent cells	10 reactions
GE-SCT7-1212	StabyCodon T7 expression kit, chimio-competent cells	10 reactions
Staby™Switch		
GE-AIME-04	Auto-induction medium	2L
Cherry™Express		
GE-CET7-05	CherryExpress T7 expression kit, electrocompetent	5 reactions
GE-CET7-07	CherryExpress T7 expression kit, chimio-competent cells	5 reactions
GE-CET7-10	CherryExpress T7 expression kit, electrocompetent	10 reactions
GE-CET7-12	CherryExpress T7 expression kit, chimio-competent cells	10 reactions
Cherry™Codon		
GE-CCT7-05	CherryCodon T7 expression kit, electrocompetent	5 reactions
GE-CCT7-07	CherryCodon T7 expression kit, chimio-competent	5 reactions
GE-CCT7-10	CherryCodon T7 expression kit, electrocompetent	10 reactions
GE-CCT7-12	CherryCodon T7 expression kit, chimio-competent	10 reactions

Alpha-numeric grid for tubes identification:

Researcher name:

Date:

Product reference:

DG1 lot number:

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Worldwide ordering:

You can order our products directly from Delphi Genetics worldwide (with the exception of Japan) using our online ordering platform (www.delphigenetics.com). Should you prefer to work with a local dealer, you can find a list of distributors on our website: <http://www.delphigenetics.com/international-distributors.html>

Contact us:



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