

Staby™ Switch

Auto-inducible medium for protein expression

Instruction manual (v2.1)



Table of contents

• Content and storage	2
• Material Safety and Data Sheet	2
• User Guide	3
○ Overview of the Staby™Switch auto-inducible medium	3
○ Benefits of the Staby™Switch medium	4
○ The 2 steps of the Staby™Switch system	4
▪ Step 1. Culture and expression using Staby™Switch	5
▪ Step 2. Protein extraction	6
○ Troubleshooting	7
• References	7
• Related Staby™ products and services	8
• Staby™ products ordering information	9
• Worldwide ordering	10

Content and storage :

The *Staby™Switch* auto-inducible medium is shipped at room temperature.

Storage: from 4°C to 25°C. Protect from moisture. The bottles containing the medium must be closed tightly.

Each box contains the following items:

Reference	Amount	Final volume of medium
GE-AIME-04	2 bottles containing each 1l of Staby™Switch auto-inducible medium (sterile) + manual	2 x 1l = 2 l

Material Safety Data Sheet:

Producing Company identification:

Delphi Genetics SA
Rue C. Ader, 16
B-6041 Charleroi, Belgium

Tel: +32.71.37.85.25
Fax: +32.71.37.60.57
e-mail: delphigenetics@delphigenetics.com

Hazards identification

There is no specific hazard concerning the products of the Staby™Switch auto-inducible medium.

First aid measures

- Inhalation: If one of the products of the Staby™Switch auto-inducible medium 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 Staby™Switch auto-inducible medium 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 Staby™Medium kit, 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

Overview of the *Staby™Switch* auto-inducible medium:

The *Staby™Switch* medium is designed for high-level protein expression using *Staby™* products (*StabyExpress™* or *Staby™Codon*) or any other IPTG-inducible bacterial expression system (Lac or Tac promoters, T7 expression systems based on the use of SE1 or BL21(DE3) bacteria,...). *Staby™Switch* is an auto-inducible medium: metabolization of medium components during bacterial growth will automatically induce protein expression at high cell density. Thus, it is neither necessary to add IPTG (isopropyl- β -D-thiogalactoside) nor to monitor optical density during bacterial growth. Furthermore, the target protein yield is often higher than using conventional IPTG induction. The best results are obtained after 24 hours of culture at 37°C. It is therefore easy to express protein during an overnight culture.

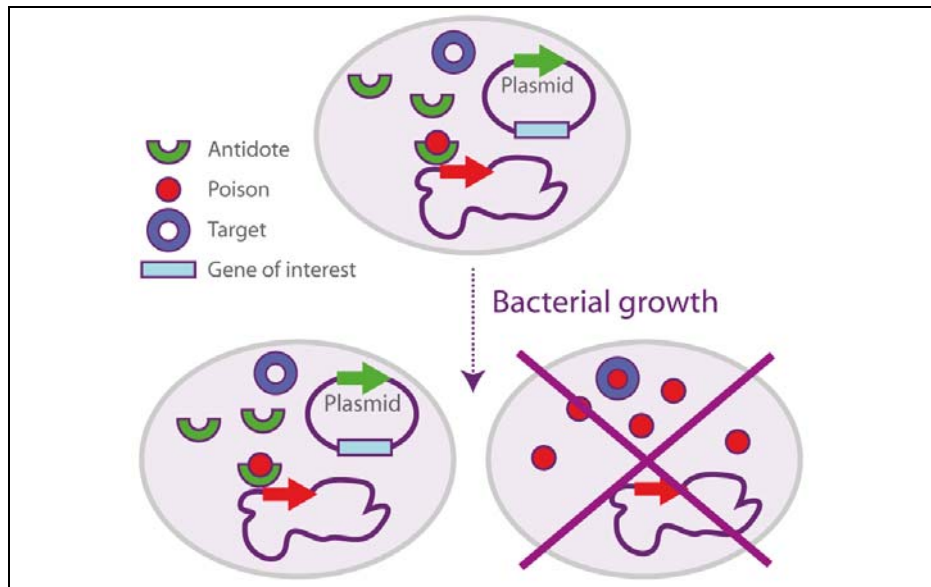
The *Staby™Switch* auto-inducible medium is a complete culture medium supplied as a ready-to-use liquid medium. Each bottle contains 1 liter of sterile culture medium. The sterile medium is stable for several months when stored at room temperature or 4°C (see expiration date on label)

When using an auto-inducible medium, bacteria will grow during several hours before and during induction. If some bacteria without plasmid-vector appear during the growth, they will grow faster than the bacteria carrying the plasmid because plasmid replication and transcription, as well as protein production represent a significant burden on cellular metabolism. Most often, the use of antibiotics in intensive culture conditions (high biomass, continuous culture, protein expression) can reduce but not solve this problem. Consequently, bacteria without plasmid-vector will rapidly overcome the plasmid-bearing cells and the protein-production yield will fall. **When using an auto-inducible medium, it is essential to completely stabilize the plasmid encoding the protein of interest to obtain high expression yield.** It is thus strongly recommended to use *Staby™* products (*StabyExpress™*, *Staby™Codon*) for high-yield protein expression. In these products, the antidote gene (*ccdA*) is localized in the plasmid DNA under the control of a constitutive promoter. On the other hand, the toxic gene (*ccdB*) is localized in the chromosome of the bacteria (cf. figure 1) and its expression is under the control of a promoter strongly repressed in the presence of the plasmid. When the plasmid is lost, the antidote is degraded and the production of the toxin is induced, causing cell death. If some bacteria lose the vector, they will not obtain a selective (growth speed) advantage, but will die. In practice, this means that during bacterial growth, 100% of the bacteria will carry the vector of interest.

For manufacturers of recombinant DNA or proteins, this system offers a great benefit because it is an antibiotic-free system. Therefore the manufactured product will also be free of any trace of antibiotics.

When the *Staby™* cassette (the DNA fragment encoding the antidote protein) is added to a vector, this vector is stabilized in any strain containing the *ccdB*-poison gene in its chromosome. It is easy to add the *Staby™* cassette to your vector using the *GetStaby™* kit.

Figure 1: Principle of the stabilization system



Benefits of the *Staby™Switch* medium:

- Auto-induction of the protein expression;
- High yield of protein production using *Staby™Switch* systems or other IPTG-inducible expression systems;
- No IPTG required;
- Ready-to-use (no autoclaving required)
- Culture and expression do not require monitoring bacterial growth;
- Overnight expression directly from a single colony or a glycerol stock;

The 2 steps of the *Staby™Switch* system:

1) Inoculate the desired culture volume with a single colony or a glycerol stock and incubate with shaking (37°C, 24 hours, 200rpm)



2) Centrifuge and extract proteins

Step1: Culture and expression using *Staby™Switch* auto-inducible medium

The conditions described below were developed for *Staby™* kits (*StabyExpress™*, *Staby™Codon*, *Cherry™Express*, *Cherry™Codon*) using the SE1 strain. The *Staby™Switch* medium can be used with other strains and other expression systems (IPTG-inducible systems) but the conditions may require optimization. In all cases, it is necessary to take into account the following parameters: target protein, host strain, temperature, and culture volume.

Note: Each bottle contains 1 liter of sterile culture medium. This medium is ready-to-use. Optionally, you can aliquot the medium in sterile containers. Generously fill the containers to avoid leaving a large volume of air on top of the medium. Do not autoclave the medium after aliquoting (the medium composition could be modified when medium is autoclaved). Store at room temperature for 2 days, check for the absence of contaminants and store at 4°C or room temperature. The sterile medium is stable for several months (see expiration date on label).

Protocol:

1. Inoculate two containers containing the desired volume of pre-warmed *Staby™Switch* medium with a few microliters (1 or 2 µl / 10ml culture) from a glycerol stock of the strain carrying the gene to express (SE1 strain in the case of *Staby™* kits). Alternatively, inoculate containers with a single colony from a plate streaked with the bacteria containing your construction (SE1 strain in the case of *Staby™* kits).

For a 10ml culture volume, the use of 50ml tubes with conical bottom (28mm x 114mm) is ideal. The tubes can be maintained closed during all the whole expression experiment. For bigger culture volumes, use Erlenmeyer flasks with a capacity of 5 times the culture volume. For 96 well plates, use 1 single colony or 0.001 volume of a glycerol stock per well.

2. Add the appropriate antibiotics or no antibiotics if you are using the *Staby™* system.
3. Add 1% sterile glucose (from a sterile-filtered 20% stock solution) to one of the two containers. This culture will be used as a non-induced control and/or to prepare a glycerol stock.
4. Incubate the containers at 37°C for approximately 24 hours with shaking (200rpm max, rotary shaker, 2.54cm orbit).

Note: (1) If your protein is unstable, add 1% lactose (from a sterile-filtered 20% stock solution) 2 hours before the end of the culture.

(2) It is essential to grow the bacteria to stationary phase for full induction. If you want to incubate your cultures at lower temperature (<37°C), it is necessary to adapt the incubation time. Continue incubation for several hours (8 to 10 hours) after saturation. The first time, it is recommended to take a sample every hour and to check the protein expression on a SDS-PAGE gel. You can also use the *Cherry™* system for an easy visual check of the induction efficiency (see documentation about *Cherry™Express* and *Cherry™Codon* kits).

Step2: Protein extraction

Protein extraction protocols already established using other expression conditions can be readily used with the *Staby™Switch* cultures.

Protocol for a small-scale analysis:

The small-scale protocol below will allow you to verify that the target protein is produced and to verify for the presence of detection tags in the target protein.

1. For each culture, transfer a 1ml sample into a microcentrifuge tube. Add 50µl of cold 100% Trichloroacetic acid (TCA) (w/v) to each sample and vortex for a few seconds.
Note: The TCA precipitation protocol allows for the analysis of the total protein content of the cells. Other methods can be used to specifically analyze different fractions (soluble, insoluble, periplasm, ...) in order to identify the cellular localization of the target protein. For more information, please, check specialized literature or protocols (e.g., Sambrook et al., Ausubel et al.).
2. Place on ice for 10min.
3. Centrifuge at maximum speed (13000 g) for 10 min (if possible at 4°C).
4. Remove carefully and discard the supernatant.
5. Wash the pellet with cold acetone (+4°C): add 500µl of acetone, vortex, and centrifuge for 5 min at maximum speed (if possible at 4°C).
6. Repeat steps 4 and 5.
7. Remove carefully and discard the supernatant. Air dry the pellet: leave the tube opened on the bench or use vacuum drying.
8. Add 300µl of 1X sample buffer (2X sample buffer= 100mM DTT, 2% SDS, 80mM Tris-HCl, pH 6.8, 0.006% bromophenol blue, 15% glycerol). Vortex vigorously to resuspend the pellet.
9. Heat the samples at 70°C-100°C (10min.) to resuspend and denature the proteins. The samples can be used directly for SDS-PAGE analysis or stored at -20°C.
10. Load 4 to 10 µl of each sample on a SDS-PAGE gel containing the appropriate concentration of polyacrylamide (according to the size of the overproduced protein). Add a molecular size marker.
Note: The sample volume that needs to be loaded will depend on the gel size, the expression level, and the extraction efficiency.
11. After migration, visualize the proteins with Coomassie-blue staining or continue the analysis with western blot. Compare the results obtained with the induced culture and the non-induced control (containing 1% glucose).
Note: Western blot analysis is a more specific and sensitive method but requires protein-specific antibodies or fusion tag-specific antibodies. For more information, please, check specialized literature or protocols (e.g., Sambrook et al., Ausubel et al.).
12. Optional: prepare a glycerol stock: Mix well 800µl of the non-induced control (containing 1% glucose) with 800µl of sterile glycerol and transfer to a cryovial. Store at -80°C.

Troubleshooting:

Most problems of expression can be solved as described in the table below. However, due to intrinsic and specific properties of your protein of interest, the efficiencies of the proposed solutions may vary.

Problem	Solution
No or Low amount of protein of interest.	<p>-Check the plasmid stability or use the StabyExpress™ or Staby™Codon kits. In these kits, the plasmid is completely stabilized by our stabilization technology (even in the absence of antibiotics). Plasmid instability happens frequently when ampicillin is used alone for plasmid selection.</p> <p>-Check your plasmid construction by sequencing. Check the open-reading-frame of the gene-of-interest. Check the position of the ribosome binding site.</p> <p>-Check expression starting with a single colony or a glycerol stock (do not use pre-culture)</p> <p>-Pre-warm the medium at room temperature or 37°C before inoculation.</p> <p>-Check for the presence of rare codons. If several rare codons are present, it is recommended to use Staby™Codon kit. Please, contact us for an analysis of your gene-of-interest.</p> <p>-Check for the presence of the pLys plasmid. When using a plasmid vector with a T7lac promoter (as pStaby1.2, pSCodon1,...) , it is recommended to use a host without pLys plasmid (such as SE1 or BL21 (DE3)). The use of the pLys plasmid will reduce expression levels when using Staby™Switch medium.</p> <p>-Check the genotype of the strain used. The presence of mutations in the <i>lacY</i>, <i>lacZ</i> genes can affect the auto-induction level. It is recommended to use the SE1 or BL21 (DE3) strains.</p> <p>-If you express the protein for the first time or if you incubate the culture at temperature lower than 37°C, take samples every hour after culture saturation and analyze the expression on SDS-PAGE gel to determine the best incubation time or use the Cherry™ system for visual check of the induction efficiency (see documentation about Cherry™Express or Cherry™Codon kits).</p> <p>-Check the shaking speed: use max 200rpm (rotary shaker, 2.54cm orbit).</p> <p>-It might be that your protein is unstable. Add 1% lactose (from a sterile-filtered 20% stock solution) 2 hours before the end of the culture.</p> <p>-After long storage of dissolved Staby™Switch medium, check the pH. The ideal pH is 6.5. adjust the pH if necessary (with sterile HCl or NaOH) or use fresh medium.</p> <p>-Medium autoclaved after aliquoting. The Staby™Switch medium is sterile and ready-to-use. Do not autoclave the medium. If you want to aliquot the medium, use sterile containers.</p>
Contaminants in the medium after aliquoting.	Check the method used to aliquot the medium. Use sterile containers. Do not autoclave after aliquoting (the medium composition could be changed when the medium is autoclaved).
No bacterial growth	Check the antibiotics used in the culture: Check the medium with another strain which is resistant to the same antibiotic. If no growth is observed, check your antibiotic solution.

References:

- Ausubel F., Brent R., Kingston R., Moore D., Seidman J.G., Smith J.A., Struhl K. 1995. Current Protocols in Molecular Biology. John Wiley and Sons edition. USA.
- Sambrook J., Fritsch E., Maniatis T. 1989. in Molecular Cloning: a laboratory manual, Second edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

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

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:



DELPHI
genetics

Delphi Genetics SA
Rue C. Ader, 16
B-6041 Charleroi
Belgium

Tel: +32.71.37.85.25

Fax: +32.71.37.60.57

www.delphigenetics.com

delphigenetics@delphigenetics.com