USER GUIDE

invitrogen
by life technologies

ChargeSwitch® Plasmid ER Mini Kit

For purification of plasmid DNA (endotoxin-reduced) from bacterial cells

Catalog no. CS10100

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For Research Use Only. Not for diagnostic procedures.

life technologies

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Kit Contents and Storage

Shipping and Storage

All components of the ChargeSwitch® Plasmid ER (Endotoxin Reduced) Mini Kit are shipped at room temperature. Upon receipt, store components as follows:

- Store Precipitation Buffer (N5) at 4°C
- Mix RNase A in Resuspension Buffer (R4) as described on page 8 and store RNase A in Resuspension Buffer (R4) at 4°C
- Store the remaining components at room temperature

All components are guaranteed stable for 6 months when stored properly.

Contents

The components supplied in the ChargeSwitch® Plasmid ER Mini Kit are listed below. The reagents supplied are sufficient to perform 50 purifications.

Component	Amount
ChargeSwitch® Magnetic Beads (25 mg/ml) in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20	2 x 1 ml
RNase A (5 mg/ml in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	0.4 ml
Resuspension Buffer (R4; 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	15 ml
ChargeSwitch® Lysis Buffer (L9)	15 ml
ChargeSwitch® Precipitation Buffer (N5)	15 ml
ChargeSwitch® Wash Buffer (W11)	50 ml
ChargeSwitch® Wash Buffer (W12)	50 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	10 ml
Endotoxin Reduction Reagent (D1; ETRR)	4.5 ml

Product Qualification

Functional Qualification

Each kit is functionally tested to ensure conformance with the most current approved product specifications.

Current specifications consist of tests for:

- Bead size, charge, and binding capacity
- Nucleic acid quality and quantity
- Buffer turbidity, volume, and absence of RNases and DNases
- Kit packaging and labeling accuracy

For individual lot test results and more information, visit www.invitrogen.com to download the Certificate of Analysis.

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® Plasmid ER Mini Kit.

A large selection of ChargeSwitch® products are available from Invitrogen for plasmid and genomic DNA purification from various sources. For more information, visit www.invitrogen.com or contact Technical Service (page 15).

Product	Amount	Catalog no.
MagnaRack™ Magnetic Rack	1 rack	CS15000
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit, 1 ml	P7589
Luria Broth Base (Miller's LB Broth Base)®, powder	2.5 kg	12795-084
Ampicillin	200 mg	11593-019
Carbenicillin, Disodium Salt	5 g	10177-012
ChargeSwitch® NoSpin Plasmid Micro Kit	96 reactions	CS10201
	960 reactions	CS10201-10
ChargeSwitch® NoSpin Plasmid Mini Kit	50 reactions	CS10200
ChargeSwitch® gDNA Micro Tissue Kit	50 reactions	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 reactions	CS11204
ChargeSwitch® gDNA 1 ml Blood Kit	20 reactions	CS11001
ChargeSwitch® gDNA 1 ml Serum Kit	50 reactions	CS11040

E-Gel® Agarose Gels and DNA Ladders

E-Gel® Agarose Gels are bufferless pre-cast agarose gels designed for fast, convenient electrophoresis of DNA samples. E-Gel® agarose gels are available in different agarose percentage and well formats for your convenience.

A large variety of DNA ladders are available from Invitrogen for sizing DNA.

For more details on these products, visit our website at www.invitrogen.com or contact Technical Service (page 15).

Introduction

Overview

Introduction

The ChargeSwitch® Plasmid ER (Endotoxin Reduced) Mini Kit allows rapid and efficient purification of highly pure plasmid DNA from *E. coli* cells.

After preparing cell lysates using the modified alkaline lysis procedure, the plasmid DNA is purified from lysates in less than 10 minutes using the ChargeSwitch® Technology. For more information on the Charge Switch® Technology, see below.

The kit includes Endotoxin Reduction Reagent (ETRR) that substantially lowers the endotoxin levels (<50 EU/µg DNA) in the purified plasmid DNA.

The isolated plasmid DNA is suitable for a variety of downstream applications such as mammalian transfection, automated fluorescent DNA or manual sequencing, PCR, cloning, *in vitro* transcription, bacterial cell transformations, and restriction digestion.

The ChargeSwitch® Technology

The ChargeSwitch® Technology is a novel magnetic bead-based technology that provides a switchable surface which is charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification.

In low pH conditions, the ChargeSwitch® Magnetic Beads have a positive charge that binds the negatively charged nucleic acid backbone (see figure below). Proteins and other contaminants are not bound and are simply washed away in aqueous wash buffers. To elute nucleic acids, the charge on the surface is neutralized by raising the pH to 8.5 using a low salt elution buffer (see figure below). Purified DNA elutes instantly into this elution buffer, and is ready for use in downstream applications of choice.

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Overview, Continued

Advantages

Use of the ChargeSwitch® Plasmid ER Mini Kit provides the following advantages:

- High quality, high yield (up to 20 µg) plasmid DNA purification from *E. coli* using a magnetic bead-based technology without the use of chaotropic salts, organic solvents, or ethanol
- Designed to isolate plasmid DNA from samples in ~10 minutes following sample preparation
- High-quality purified plasmid DNA suited for mammalian transfections
- Reliable performance of the purified plasmid DNA in a variety of applications, including mammalian cell transfection, automated and manual sequencing, amplification reactions, *in vitro* transcription, bacterial cell transformation, cloning, and labeling.

System Specifications

Starting Material:	1-5 ml fresh, overnight LB culture
Bead Binding Capacity:	1 mg beads bind ~25 µg plasmid DNA
Bead Size:	1 µm
Bead Concentration:	25 mg/ml
Bead Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20
Elution Volume:	50-100 µl
DNA Yield:	Up to 20 µg

Methods

General Information

Introduction

Review the information in this section before starting. Guidelines are included for growing the bacterial culture.

Bacterial Cultures

Grow transformed *E. coli* in LB medium with the appropriate antibiotic. If desired, you may use richer medium like Terrific Broth to grow the *E. coli.*

Use 1-5 ml overnight bacterial cultures with an absorbance at 600 nm (A_{600}) of 9 OD units.

To obtain the best results, use fresh, overnight cultures but the kit can also be used to purify plasmid DNA from frozen cell pellets.

Follow the recommendations below to obtain the best results:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases
- Ensure that no DNase is introduced into the solutions supplied with the kit
- Make sure that all equipment coming in contact with DNA is sterile, including pipette tips and tubes
- Perform the centrifugation step during cell lysis at 4°C to improve the efficiency of the precipitation reaction (page 8)
- Perform the recommended wash steps during purification to obtain the best results

Continued on next page

MagnaRack™

You will need a magnetic rack (separator) for use with the ChargeSwitch® Plasmid ER Mini Kit. We recommend using the MagnaRack™ Magnetic Rack to obtain the best results. Other magnetic separators may not provide similar magnetic strength or may not be compatible with the volumes used in the protocol.

The MagnaRack™ Magnetic Rack available from Invitrogen (catalog no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads. The MagnaRack™ consists of a magnetic base station and a removable tube rack. The tube rack fits onto the magnetic base station in two different positions associating the row of 12 neodymium magnets with a single row of 12 tubes for simple and easy 'on the magnet' and 'off the magnet' sample processing (see figure below). For more information, see www.invitrogen.com or contact Technical Service (page 15).

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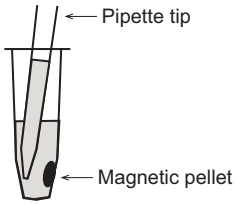
Handling Magnetic Beads

Follow the recommendations below for best results:

- During the mixing and washing steps of the ChargeSwitch® Magnetic Beads, mix beads by pipetting up and down gently to avoid forming bubbles as directed in the protocol.
- Do not allow the beads to dry as drying reduces the bead binding efficiency.
- To aspirate the supernatant after bead washing, place the pipette tip away from the beads by angling the pipette such that the tip is pointed away from the pellet and carefully remove the supernatant without disturbing or removing any beads (see figure below).



- **Do not freeze the magnetic beads** as freezing damages the beads and cannot be used for nucleic acid purifications.

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General Information, Continued

Elution Buffer

The plasmid DNA is eluted with Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5). To obtain the best results, always use Elution Buffer (E5) to elute the DNA. If you wish to elute the plasmid DNA in any other buffer, be sure to use a buffer of **pH 8.5-9.0**. If the pH of the buffer is <8.5, the DNA will not elute.

Do not use water for elution.

The plasmid DNA is eluted using 50-100 µl elution buffer. The volume of elution buffer can be changed to obtain plasmid DNA in the desired final concentration. To obtain the best results, always use a volume of elution buffer that is equal or greater than the volume of beads used in the protocol. If the volume of elution buffer is lower than the volume of beads, DNA elution is incomplete and you may need to perform a second elution to recover all DNA.

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® Plasmid ER Mini Kit.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- **Do not** add bleach or oxidizing agents directly to the sample preparation waste
- If a spill of the buffers occurs, clean with a suitable laboratory detergent and water. If the liquid spill contains potentially infectious agents, clean the affected area first with laboratory detergent and water, then with 1% (v/v) sodium hypochlorite or a suitable laboratory disinfectant.

Isolating Plasmid DNA

Introduction

Instructions for isolating plasmid DNA from 1-5 ml overnight bacterial culture are described below.

The procedure is designed for isolating plasmid DNA using a ChargeSwitch® Magnetic Beads procedure in a total time of **~10 minutes** after sample preparation.

To obtain high-quality, low endotoxin level plasmid DNA, use the Endototxin Reduction Reagent (ETRR) as recommended on page 9.

Materials Needed

- 1-5 ml overnight bacterial culture (page 3)
- MagnaRack™ Magnetic Rack (page 4)
- Sterile 1.5 ml microcentrifuge tubes
- Adjustable pipettes and aerosol barrier pipette tips

Components supplied with the kit

- RNase A in Resuspension Buffer (R4), see next page
- ChargeSwitch® Precipitation Buffer (N5)
- ChargeSwitch® Lysis Buffer (L9)
- Endotoxin Reduction Reagent (D1)
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Wash Buffer (W11)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5)

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Isolating Plasmid DNA, Continued

Before Starting

Resuspension Buffer (R4)

Add the entire content of supplied RNase A to the Resuspension Buffer (R4). Mix well. Mark the bottle label to indicate that RNase A is added. Store the buffer with RNase at 4°C.

Precipitation Buffer (N5)

Chill an aliquot of the Precipitation Buffer (N5) to 4°C.

Lysis Buffer (L9)

Check the Lysis Buffer (L9) for precipitates. If present, warm the solution briefly at 37°C to dissolve the precipitate.

Preparing Bacterial Lysates

1. Harvest 1-5 ml overnight bacterial LB culture (page 3) by centrifugation at room temperature.
2. Resuspend the cell pellet in 300 µl Resuspension Buffer (R4) containing RNase A, above. Pipet up and down to completely resuspend the pellet.
3. Add 300 µl Lysis Buffer (L9) to the tube and mix by inverting the capped tube 6 times.
4. Incubate at room temperature for 2-5 minutes. **Do not** incubate for longer than 5 minutes.
5. Add 300 µl chilled Precipitation Buffer (N5) and mix by inverting the capped tube until a white, free-flowing precipitate is formed.
6. Centrifuge the tube in a microcentrifuge at maximum speed for 10 minutes at 4°C.
7. Transfer the clear supernatant to a new sterile microcentrifuge tube.
8. Proceed immediately to **Binding DNA**, next page.

Continued on next page

Isolating Plasmid DNA, Continued

Binding DNA

Follow the procedure below to bind DNA to the ChargeSwitch® Magnetic Beads.

1. Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend and evenly distribute the beads in the storage buffer.
2. **Optional:** Add 90 µl Endotoxin Reduction Reagent (D1) to the samples if low endotoxin level is desirable.
3. Add 40 µl of ChargeSwitch® Magnetic Beads to the lysate.
4. Pipet up and down gently to mix without forming bubbles.
5. Incubate at room temperature for 1 minute to allow the DNA to bind to the beads.
6. Place the sample on the MagnaRack™ Magnetic Rack for 1 minute or until the beads have formed a tight pellet.
7. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant without disturbing the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 5).
8. Proceed immediately to **Washing DNA**, next page.

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Isolating Plasmid DNA, Continued

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 7, previous page). There should be no supernatant in the tube.
2. Add 1 ml Wash Buffer (W11) to the tube and pipet up and down gently to mix the sample without forming bubbles.
3. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
4. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
5. Add 1 ml Wash Buffer (W12) to the tube and pipet up and down gently to mix the sample without forming bubbles.
6. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
7. Without removing the tube from the MagnaRack™, carefully remove and discard the supernatant. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.
8. Proceed immediately to **Eluting DNA**, next page.

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Isolating Plasmid DNA, Continued

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™. There should be no supernatant in the tube.
2. Add 50-100 µl of Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) to the tube and pipet up and down gently 5-10 times to mix the sample without forming bubbles.

   Note: See page 6 for more information on elution buffer volume.
3. Incubate at room temperature for 1 minute.

   Tip: For maximum yield, mix the suspension of beads (by pipetting up and down gently) half way through the incubation.
4. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
5. Without removing the tube from the MagnaRack™, carefully remove the **supernatant containing the DNA** to a sterile microcentrifuge tube. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet.

   Note: If the supernatant containing the DNA is discolored, repeat Steps 4-5.
6. Discard the used magnetic beads. **Do not** re-use the magnetic beads.

Storing DNA

- Store the purified DNA at -20°C or use DNA for the desired downstream application.
- To avoid repeated freezing and thawing of DNA, store the purified DNA at 4°C for immediate use or aliquot the DNA and store at -20°C for long-term storage.

Analyzing Plasmid DNA Yield and Quality

Plasmid DNA Yield

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution in 10 mM Tris-HCl, pH 7.5. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against 10 mM Tris-HCl pH 7.5
2. Calculate the concentration of DNA using the formula:

   DNA (µg/ml) = $A_{260} \times 50$ x dilution factor

   For DNA, A_{260} = 1 for a 50 µg/ml solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ Kits

The Quant-iT™ Kits (see page vi for ordering information) provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. The kit contains a state-of-the-art quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers or fluorometers.

Plasmid DNA Quality

Typically, plasmid DNA isolated using the ChargeSwitch® Plasmid ER Mini Kit has an A_{260}/A_{280} ratio 1.7 when samples are diluted in Tris-HCl pH 7.5, indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Absence of contaminating RNA may be confirmed by agarose gel electrophoresis.

Troubleshooting

Introduction

Refer to the table below to troubleshoot problems that you may encounter when purifying plasmid DNA with the kit.

Problem	Cause	Solution
Low plasmid DNA yield	Poor quality of starting material or incomplete lysis	• Check the growth conditions of the cell culture to ensure plasmid propagation. Use a high copy number plasmid if possible. • If the cell lysate is too viscous, reduce the amount of cells used per sample. • Ensure complete resuspension of the bacterial cell pellet. Decrease the amount of starting material used. • Use Precipitation Buffer (N5) chilled to 4°C and perform centrifugation at 4°C to improve the precipitation efficiency and plasmid DNA yield. • Increase the incubation time during lysis but do not exceed 5 minutes.
	Incorrect elution conditions	• After adding Elution Buffer (E5) to the sample, pipet up and down to resuspend the magnetic beads before incubation. • Increase the mixing steps during elution to 20-30 times. • If you are using a buffer for elution, ensure the pH of the buffer is 8.5-9.0.

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Troubleshooting, Continued

Problem	Cause	Solution
Low plasmid DNA yield	Magnetic beads not functional	**Do not freeze the magnetic beads.** Store the magnetic beads at room temperature. Do not re-use the magnetic beads.
	Incorrect magnetic bead amount used for binding	Use the recommended amount of magnetic beads for binding. If plasmid DNA yields are lower, you may increase the bead amount used and increase the volume of wash and elution buffer proportionally.
Eluate containing plasmid DNA is discolored	Magnetic pellet disturbed during elution	Place the sample in the MagnaRack™ until the beads form a tight pellet. Remove the eluate to a sterile microcentrifuge tube, taking care not to disturb the bead pellet.
Genomic DNA contamination	Genomic DNA sheared during handling	Gently invert tubes to mix after adding buffers. **Do not vortex** as it can shear genomic DNA. To efficiently precipitate the genomic DNA away from the plasmid DNA, the genomic DNA must be intact.
Plasmid DNA degradation	Incorrect lysis procedure	Incubate the lysate at room temperature for no longer than 5 minutes.
	Bubbles formed during mixing steps	Make sure that the pipette tip is submerged in the solution during mixing.

Appendix

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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MSDS Requests

To request an MSDS, visit our Web site at **www.invitrogen.com.** On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

Purchaser Notification

License

Limited Use Label License No. 265: ChargeSwitch® Technology

The use of this product may be covered by European Patent No. EP1036082B1 and foreign equivalents.

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Service Representatives. Invitrogen warrants that all of its products will perform according to specifications stated on the certificate of analysis. The company will replace, free of charge, any product that does not meet those specifications. This warranty limits Invitrogen Corporation's liability only to the cost of the product. No warranty is granted for products beyond their listed expiration date. No warranty is applicable unless all product components are stored in accordance with instructions. Invitrogen reserves the right to select the method(s) used to analyze a product unless Invitrogen agrees to a specified method in writing prior to acceptance of the order. Invitrogen makes every effort to ensure the accuracy of its publications, but realizes that the occasional typographical or other error is inevitable. Therefore Invitrogen makes no warranty of any kind regarding the contents of any publications or documentation. If you discover an error in any of our publications, please report it to our Technical Service Representatives. **Invitrogen assumes no responsibility or liability for any special, incidental, indirect or consequential loss or damage whatsoever. The above limited warranty is sole and exclusive. No other warranty is made, whether expressed or implied, including any warranty of merchantability or fitness for a particular purpose.**

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