USER GUIDE

invitrogen™
by life technologies™

ChargeSwitch® NoSpin Plasmid Kits

For purification of plasmid DNA from bacterial cells using the MagnaClear™ Technology

Catalog nos. CS10200, CS10201, CS10201-10

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Kit Contents and Storage

Types of Kits

This manual is supplied with the following products.

Product	Number of Purifications	Catalog no.
ChargeSwitch® NoSpin Plasmid Mini Kit	50	CS10200
ChargeSwitch® NoSpin Plasmid Micro Kit	96	CS10201
	960	CS10201-10

Shipping and Storage

All components of the ChargeSwitch® NoSpin Plasmid Kits are shipped at room temperature. Upon receipt, store as follows:

- Store ChargeSwitch® Precipitation Buffer (N5) at 4°C.
- Mix the RNase I in ChargeSwitch® Resuspension Buffer (R4) and store the resulting solution at 4°C. For details, see pages 12, 18, and 26 as appropriate.
- Store all other components at room temperature.

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

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Kit Contents and Storage, continued

Contents

The components supplied in the ChargeSwitch® NoSpin Plasmid Kits are listed below.

Note: Some reagents in the kit are provided in excess of the amount needed.

Components	Catalog no.		
	CS10200	**CS10201**	**CS10201-10**
ChargeSwitch® MagnaClear™ Beads	1.5 ml	3 ml	30 ml
ChargeSwitch ® Magnetic Beads	1.5 ml	2 x 1 ml	2 x 10 ml
ChargeSwitch® Resuspension Buffer (R4; 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	20 ml	20 ml	125 ml
RNase A (5 mg/ml in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)	0.4 ml	0.4 ml	2.5 ml
ChargeSwitch® Lysis Buffer (L9)	15 ml	10 ml	100 ml
ChargeSwitch® Precipitation Buffer (N5)	15 ml	10 ml	100 ml
ChargeSwitch® Wash Buffer (W11)	50 ml	20 ml	200 ml
ChargeSwitch® Wash Buffer (W12)	50 ml	20 ml	200 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	5 ml	10 ml	100 ml

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® NoSpin Plasmid Kits. In addition, the table lists a selection of ChargeSwitch® Plasmid Kits that are available for purification of plasmid DNA from different types of cells. For more information about these and other ChargeSwitch® Plasmid Kits, refer to our Web site at www.invitrogen.com or call Technical Service (see page 32).

Product	Amount	Catalog no.
MagnaRack™	1 rack	CS15000
96-well Magnetic Separator	1 rack	CS15096
ChargeSwitch® Plasmid ER Mini Kit	50 purifications	CS10100
ChargeSwitch® Plasmid Yeast Mini Kit	50 purifications	CS10203
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130

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

For more details on these products, visit www.invitrogen.com or contact Technical Service (page 32).

Introduction

Overview

Introduction

The ChargeSwitch® NoSpin Plasmid Kits allow rapid and efficient purification of plasmid DNA from a fresh overnight culture of bacterial cells. The kit uses the MagnaClear™ Technology to allow magnetic bead-based capture of bacterial cells directly from a liquid culture. After lysing the bacterial cells, you may purify plasmid DNA in less than 15 minutes using the ChargeSwitch® Technology. Depending on the kit used, samples may be handled individually or in an automated system using a liquid handling robot. For more information about the ChargeSwitch® and MagnaClear™ Technologies, see page 3.

Intended Use for the Kits

The ChargeSwitch® NoSpin Plasmid Kits are designed to allow isolation of plasmid DNA from the following sources:

- Micro Kit: Purifies up to 5 µg of plasmid DNA from 1 ml of fresh overnight culture in a 96-well format.
- Mini Kit: Purifies up to 6 µg of plasmid DNA from 0.5-1.5 ml of fresh overnight culture.

The kits are designed for plasmid purification from fresh overnight cultures grown in LB Broth. Higher DNA yields may be obtained from cultures grown in richer media (*e.g.* Terrific Broth). Cultures with optical densities (measured at 600 nm) of up to 6 OD units can be used.

Note: The kits may be used to purify BACs, cosmids, and phagemids; however, yields may be reduced due to the relatively low concentration of these species within the cell.

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Advantages

Use of the ChargeSwitch® NoSpin Plasmid Kits to isolate plasmid DNA provides the following advantages:

- Uses the ChargeSwitch® and MagnaClear™ magnetic bead-based technologies to isolate high quality plasmid DNA without the need for hazardous chemicals, any centrifugation steps, or vacuum manifolds
- Rapid and efficient purification of plasmid DNA from bacterial cells in less than 15 minutes following lysis
- Minimal contamination with RNA
- The purified plasmid DNA demonstrates improved downstream performance in applications including mammalian cell transfection, automated and manual sequencing, PCR, bacterial cell transformation, *in vitro* transcription, cloning, and labeling
- Includes a kit designed for automated processing of large numbers of samples in 96-well plates using a liquid handling robot

System Specifications

Starting Material:	Fresh bacterial culture (1 ml for Micro Kit and 0.5-1.5 ml for Mini Kit)
Elution Volume:	Varies (20-100 µl)
DNA Yield:	Up to 5 µg (Micro Kit) or 6 µg (Mini Kit)
DNA Size:	Varies (depends on size of plasmid)

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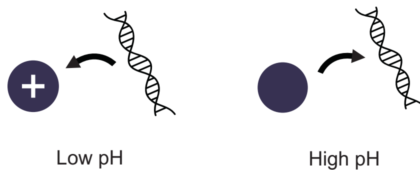
The ChargeSwitch® Technology

The ChargeSwitch® Technology (CST®) is a novel magnetic bead-based technology that provides a switchable surface charge dependent on the pH of the surrounding buffer to facilitate nucleic acid purification. In low pH conditions, the CST® 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 an aqueous wash buffer. To elute nucleic acids, the charge on the surface of the bead 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.



ChargeSwitch® Magnetic Bead Specifications

Bead Binding Capacity:	Up to 25 µg plasmid DNA per mg
Bead Size:	< 1 µm
Bead Concentration:	25 mg/ml
Storage Buffer:	10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20

MagnaClear™ Technology

The MagnaClear™ Technology is a novel magnetic bead-based technology that completely removes the need for centrifugation in plasmid purification procedures. The MagnaClear™ Beads use ChargeSwitch™ Magnetic Beads in a proprietary buffer formulation to allow capture and binding of bacterial cells directly from liquid cultures. Alkaline lysis is then performed in the presence of the MagnaClear™ Beads so that the SDS precipitate containing cell debris can be captured and removed by magnetic separation.

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Overview, continued

MagnaClear™ Magnetic Bead Specifications

Bead Binding Capacity:	> 1 x 10^9 bacterial cells per mg
Bead Size:	< 1 µm
Bead Concentration:	6.25 mg/ml
Storage Buffer:	Proprietary

Automated Liquid Handling

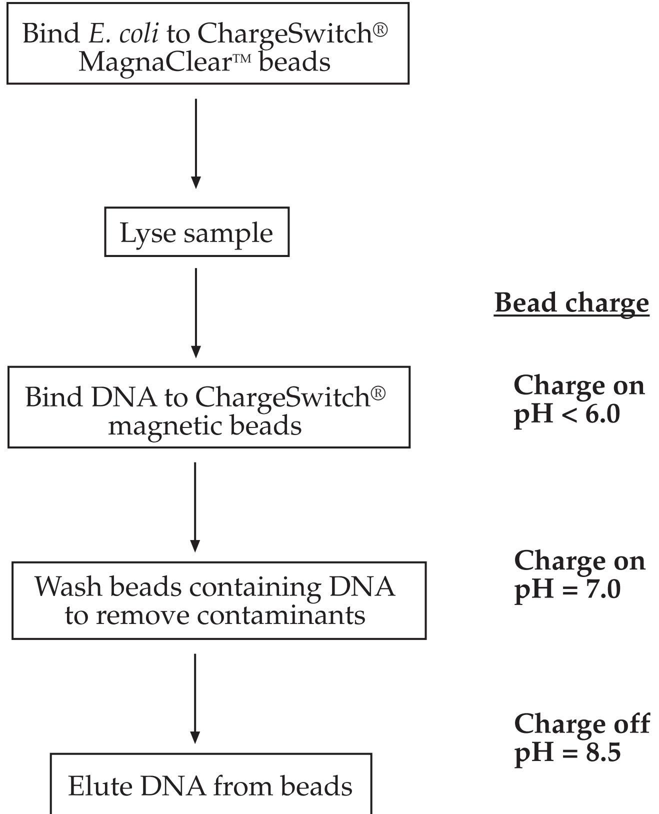
Use of the ChargeSwitch® NoSpin Plasmid Micro Kit has been demonstrated on the Tecan Genesis® robotic workstation to purify DNA in a fully automated system from bacterial cells in a 96-well format. Other liquid handling robots are suitable provided that each is equipped with a gripper arm, a 96-well magnetic separator, and other additional hardware as described on page 22. This manual provides general guidelines and a protocol that may be used to develop a script for your robot. For more information, see www.invitrogen.com or call Technical Service (page 32).

Genesis® is a registered trademark of Tecan AG Group

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify plasmid DNA from your bacterial culture using one of the ChargeSwitch® NoSpin Plasmid Kits.



Methods

General Information – Individual Samples

Introduction

This section provides general information needed to use the ChargeSwitch® NoSpin Plasmid Kits (Catalog nos. CS10200 or CS10201) to process individual samples. If you are using a liquid handling robot to process large numbers of samples, see **General Information – Automated Sample Processing**, page 16.

Bacterial Cultures

Follow the guidelines below to grow your bacterial cells.

- Grow transformed *E. coli* in LB Medium with the appropriate antibiotic. If desired, you may use richer media such as Terrific Broth to grow the *E. coli*.
- Use overnight bacterial cultures with an optical density at 600 nm (A_{260}) of up to 6 OD units.
- To obtain the highest DNA yield, purify plasmid DNA from fresh overnight cultures.

User Supplied Materials

In addition to the reagents supplied with the kit, you need to have the following materials on hand before beginning:

- A magnetic separation rack suitable for use with 1.5 ml microcentrifuge tubes (MagnaRack™; see next page) or 96-well plates (96-well Magnetic Separator; see next page)
- Sterile, 1.5 ml microcentrifuge tubes
- 96 x 2 ml deep well plate (Greiner, Catalog no. 780270 or Abgene, Catalog no. AB-0932; if using the Micro Kit)
- 96 x 300 µl U-bottomed microtiter plates (Greiner, Catalog no. 650201)
- Shaker
- Vortex mixer
- 20 µl, 200 µl, and 1 ml sterile, pipette tips

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General Information – Individual Samples, continued

MagnaRack™

The MagnaRack™ available from Invitrogen (Catalog no. CS15000) is a two-piece magnetic separation rack for use in protocols with magnetic beads, and consists of a magnetic base station and a removable tube rack. The tube rack can hold up to 24 microcentrifuge tubes. 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 'on the magnet' and 'off the magnet' sample processing (see figure below). For more information, see www.invitrogen.com or call Technical Service (page 32).

96-Well Magnetic Separator

The 96-well Magnetic Separator available from Invitrogen (Catalog no. CS15096) is a magnetic separation rack that can hold up to 96 samples in a deep well plate. The deep well plate fits onto the magnetic base station, associating the array of 24 neodymium magnets with the samples for 'on the magnet' and 'off the magnet' sample processing (see figures below). For more information, see www.invitrogen.com or call Technical Service (page 32).

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General Information – Individual Samples, continued

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® NoSpin Plasmid Kits.

- Treat all reagents supplied in the kit as potential irritants.
- Always wear a suitable lab coat, disposable gloves, and protective goggles.
- 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.

Handling the ChargeSwitch® Magnetic and MagnaClear™ Beads

Follow the guidelines below when handling the ChargeSwitch® Magnetic Beads and the ChargeSwitch® MagnaClear™ Beads.

- Do not freeze the beads as this irreparably damages them. Store the beads at room temperature.
- Always keep the beads in solution. Do not allow them to dry out as this renders them non-functional.
- When using the beads, resuspend thoroughly in the storage buffer by vortexing before removal.
- Discard beads after use. Do not reuse.

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General Information – Individual Samples, continued

Elution Buffer

ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5) is supplied with the kit for eluting the DNA from the ChargeSwitch® Magnetic Beads. For best results, use Elution Buffer (E5) to elute the DNA. Alternatively, TE Buffer, pH 8.5-9.0 is acceptable. Note that the pH must be between 8.5-9.0 otherwise the DNA will not elute. **Do not use water for elution.**

The protocol recommends eluting the plasmid DNA in 20-100 µl of ChargeSwitch® Elution Buffer (E5). Vary the amount of ChargeSwitch® Elution Buffer (E5) used to obtain plasmid DNA in the desired final concentration. **For best results, always use a volume of ChargeSwitch® Elution Buffer (E5) that is equal to or greater than the volume of ChargeSwitch® Magnetic Beads used in the protocol.** If the volume of ChargeSwitch® Elution Buffer (E5) is lower than the volume of beads used, DNA elution is incomplete. You may need to perform a second elution to recover all DNA.

Isolating Plasmid DNA from Individual Samples Using the Mini Kit

Introduction

This section provides guidelines and instructions to isolate plasmid DNA from 0.5-1.5 ml of overnight bacterial cell culture using the reagents supplied in the ChargeSwitch® NoSpin Plasmid Mini Kit (Catalog no. CS10200). If you are using the ChargeSwitch® NoSpin Plasmid Micro Kit, see the procedure on page 16-21.

Starting Material

Use this procedure to isolate plasmid DNA from 0.5-1.5 ml of fresh overnight bacterial culture. You may use a culture with an OD_{600} up to 6.

Ambient Temperature

You will perform the purification procedure at ambient (room) temperature. Make sure that the ambient temperature is between 17-23°C. Alkaline lysis is adversely affected when the ambient temperature is > 23°C, resulting in lower plasmid DNA yield.

Important

Two types of magnetic beads are provided in each kit:

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads

These beads are not interchangeable. Do not substitute one type of beads for the other at each appropriate specified point in the purification procedure.

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Isolating Plasmid DNA from Individual Samples Using the Mini Kit, continued

Materials Needed

Have the following materials on hand before beginning:

- 0.5-1.5 ml of fresh overnight bacterial culture(s)
- MagnaRack™ (Catalog no. CS15000)
- Sterile, 1.5 ml microcentrifuge tubes
- Vortex mixer
- Shaker
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)

Components Supplied with the Kit

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads
- RNase A
- ChargeSwitch® Resuspension Buffer (R4)
- ChargeSwitch® Lysis Buffer (L9)
- ChargeSwitch® Precipitation Buffer (N5)
- ChargeSwitch® Wash Buffer (W11)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

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Isolating Plasmid DNA from Individual Samples Using the Mini Kit, continued

Before Starting

Perform the following before beginning:

ChargeSwitch® Resuspension Buffer (R4)

Add the entire contents of the supplied RNase A (0.4 ml) to the tube of ChargeSwitch® Resuspension Buffer (R4; 20 ml). Mix well (total volume = 20.4 ml). Mark the tube label to indicate that RNase A is added. Store the buffer with RNase A at 4°C.

ChargeSwitch® Precipitation Buffer (N5)

Chill the Precipitation Buffer (N5) at 4°C.

ChargeSwitch® Lysis Buffer (L9)

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

ChargeSwitch® MagnaClear™ Beads and ChargeSwitch® Magnetic Beads

Vortex each tube of magnetic beads to fully resuspend and evenly distribute the beads in the storage buffer.

Preparing the Bacterial Lysate

Follow the procedure below to prepare a lysate from the bacterial cells.

1. Transfer 0.5-1.5 ml of fresh overnight bacterial culture into a sterile 1.5 ml microcentrifuge tube.
2. Add 30 µl of ChargeSwitch® MagnaClear™ Beads to each sample (make sure beads are fully resuspended before addition).
3. Place sample on a shaker and shake for 1 minute at medium speed until a brown precipitate has formed.
4. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet. Proceed to the next page.

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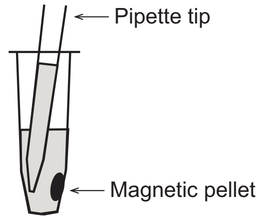
Isolating Plasmid DNA from Individual Samples Using the Mini Kit, continued

Preparing the Bacterial Lysate, continued

5. Without removing the tube from the MagnaRack™, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure below).



6. Remove the sample from the MagnaRack™ and add 300 µl of ChargeSwitch® Resuspension Buffer containing RNase A to the sample.
7. Shake the sample at top speed for 1 minute at room temperature to resuspend the pelleted beads.
8. Add 300 µl of ChargeSwitch® Lysis Buffer (L9) and shake at medium speed for 1 minute at room temperature to mix.
9. Incubate at room temperature for 2-5 minutes.
10. Add 300 µl of chilled ChargeSwitch® Precipitation Buffer (N5) and shake at top speed for 1 minute at room temperature or until a fine, grainy, brown precipitate has formed.
11. Continue shaking for 1 minute at medium speed.
12. Place the sample in the MagnaRack™ for 1 minute or until the beads have formed a tight pellet.
13. Proceed immediately to **Binding DNA**, next page.

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Isolating Plasmid DNA from Individual Samples Using the Mini Kit, continued

Binding DNA

1. Without removing the tube from the MagnaRack™, remove and transfer the supernatant to a fresh, sterile 1.5 ml microcentrifuge tube containing 30 µl of ChargeSwitch® Magnetic Beads. Pipet up and down gently twice to resuspend the magnetic beads.
2. Incubate at room temperature for 1 minute to allow DNA to bind to the beads.
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 the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure above).
5. Proceed immediately to **Washing DNA**, below.

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 4, above). There should be no supernatant in the tube.
2. Add 1 ml of ChargeSwitch® Wash Buffer (W11) to the tube and pipet up and down gently twice to resuspend the magnetic beads.

   Important: Use a 1 ml pipette tip set to 900 µl to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid 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 the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 13).
5. Repeat Steps 1-4, using ChargeSwitch® Wash Buffer (W12).
6. Proceed to **Eluting DNA**, next page.

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Isolating Plasmid DNA from Individual Samples Using the Mini Kit, continued

Eluting DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 5, previous page). There should be no supernatant in the tube.
2. Add 50-100 µl of ChargeSwitch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the tube and pipet up and down gently 10 times to resuspend the magnetic beads.

   Important: Do not use water for elution. The DNA will not elute due to the poor buffering capacity of water.
3. Incubate at room temperature for 1 minute.
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 (see figure on page 14).
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream applications.

Quantitating DNA Yield

To quantitate yield of your plasmid DNA, we recommend using one of the Quant-iT™ DNA Assay Kits available from Invitrogen (see page vii for ordering information). The Quant-iT™ kits contain a state-of-the-art quantitation reagent, pre-diluted standards, and a pre-made buffer to allow sensitive and accurate fluorescence-based quantitation of dsDNA. For more information about the Quant-iT™ DNA Assay Kits, see www.invitrogen.com or call Technical Service (page 32).

Isolating Plasmid DNA from Individual Samples Using the Micro Kit

Introduction

This section provides guidelines and instructions to isolate plasmid DNA from 1 ml of overnight bacterial cell culture in 96-well format using the reagents supplied in the ChargeSwitch® NoSpin Plasmid Micro Kit (Catalog no. CS10201). If you are using the ChargeSwitch® NoSpin Plasmid Mini Kit, see the procedure on pages 12-15.

Starting Material

Use this procedure to isolate plasmid DNA from 1 ml of fresh overnight bacterial culture. You may use a culture with an OD_{600} up to 6.

Ambient Temperature

You will perform the purification procedure at ambient (room) temperature. Make sure that the ambient temperature is between 17-23°C. Alkaline lysis is adversely affected when the ambient temperature is > 23°C, resulting in lower plasmid DNA yield.

Important

Two types of magnetic beads are provided in each kit:

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads

These beads are not interchangeable. Do not substitute one type of beads for the other at each appropriate specified point in the purification procedure.

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Isolating Plasmid DNA from Individual Samples Using the Micro Kit, continued

Materials Needed

Have the following materials on hand before beginning:

- 1 ml of fresh overnight bacterial culture(s)
- 96-Well Magnetic Separator (Catalog no. CS15096)
- 96 x 2 ml deep well culture plate
- 96 x 300 µl U-bottomed microtiter plates
- Vortex mixer
- Shaker
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)

Components Supplied with the Kit

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads
- RNase A
- ChargeSwitch® Resuspension Buffer (R4)
- ChargeSwitch® Lysis Buffer (L9)
- ChargeSwitch® Precipitation Buffer (N5)
- ChargeSwitch® Wash Buffer (W11)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

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Isolating Plasmid DNA from Individual Samples Using the Micro Kit, continued

Before Starting

Perform the following before beginning:

ChargeSwitch® Resuspension Buffer (R4)

Add the entire contents of the supplied RNase A (0.4 ml) to the tube of ChargeSwitch® Resuspension Buffer (R4; 20 ml). Mix well (total volume = 20.4 ml). Mark the tube label to indicate that RNase A is added. Store the buffer with RNase A at 4°C.

ChargeSwitch® Precipitation Buffer (N5)

Chill the Precipitation Buffer (N5) at 4°C.

ChargeSwitch® Lysis Buffer (L9)

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

ChargeSwitch® MagnaClear™ Beads and ChargeSwitch® Magnetic Beads

Vortex each tube of magnetic beads to fully resuspend and evenly distribute the beads in the storage buffer.

Preparing the Bacterial Lysate

Follow the procedure below to prepare a lysate from bacterial cells. The volumes given are on a per sample basis.

1. Transfer 1 ml of fresh overnight bacterial culture into a 96 x 2 ml deep well culture plate.
2. Add 30 µl of ChargeSwitch® MagnaClear™ Beads to each sample (make sure beads are fully resuspended before addition).
3. Place sample on a shaker and shake for 1 minute at medium speed until a brown precipitate has formed.
4. Place the sample in the 96-Well Magnetic Separator for 1 minute or until the beads have formed a tight pellet. Proceed to the next page.

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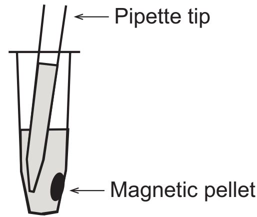
Isolating Plasmid DNA from Individual Samples Using the Micro Kit, continued

Preparing the Bacterial Lysate, continued

5. Without removing the tube from the 96-Well Magnetic Separator, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure below).



6. Remove the sample from the 96-Well Magnetic Separator and add 100 µl of ChargeSwitch® Resuspension Buffer containing RNase A to the sample.
7. Shake the sample at top speed for 1 minute at room temperature to resuspend the pelleted beads.
8. Add 100 µl of ChargeSwitch® Lysis Buffer (L9) and shake at medium speed for 1 minute at room temperature to mix.
9. Incubate at room temperature for 2-5 minutes.
10. Add 100 µl of chilled ChargeSwitch® Precipitation Buffer (N5) and shake at top speed for 1 minute at room temperature or until a fine, grainy, brown precipitate has formed.
11. Continue shaking for 1 minute at medium speed.
12. Place the sample in the 96-Well Magnetic Separator for 1 minute or until the beads have formed a tight pellet.
13. Without removing the tube from the 96-Well Magnetic Separator, remove and transfer the supernatant to a 96 x 300 µl U-bottomed microtiter plate.
14. Place the fresh plate containing the supernatant on the 96-Well Magnetic Separator for 1 minute or until the remaining beads have formed a tight pellet.
15. Proceed immediately to **Binding DNA**, next page.

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Isolating Plasmid DNA from Individual Samples Using the Micro Kit, continued

Binding DNA

1. Without removing the plate from the 96-Well Magnetic Separator, remove and transfer the supernatant to a fresh, 96 x 300 µl U-bottomed microtiter plate containing 20 µl of ChargeSwitch® Magnetic Beads. Pipet up and down gently twice to resuspend the magnetic beads.
2. Incubate at room temperature for 1 minute to allow DNA to bind to the beads.
3. Place the sample in the 96-Well Magnetic Separator for 1 minute or until the beads have formed a tight pellet.
4. Without removing the plate from the 96-Well Magnetic Separator, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 19).
5. Proceed immediately to **Washing DNA**, below.

Washing DNA

1. Remove the plate containing pelleted magnetic beads from the 96-Well Magnetic Separator (Step 4, above). There should be no supernatant in the well.
2. Add 200 µl of ChargeSwitch® Wash Buffer (W11) to the well and pipet up and down gently twice to resuspend the magnetic beads.

   Important: Use a 200 µl pipette tip set to 150 µl to mix the sample. Make sure that the tip is submerged, and pipet up and down gently to avoid forming bubbles.
3. Place the sample in the 96-Well Magnetic Separator for 1 minute or until the beads have formed a tight pellet.
4. Without removing the plate from the 96-Well Magnetic Separator, carefully remove the supernatant and discard. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 13).
5. Repeat Steps 1-4, using ChargeSwitch® Wash Buffer (W12).
6. Proceed to **Eluting DNA**, next page.

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Isolating Plasmid DNA from Individual Samples Using the Micro Kit, continued

Eluting DNA

1. Remove the plate containing the pelleted magnetic beads from the 96-Well Magnetic Separator (Step 5, previous page). There should be no supernatant in the well.
2. Add 20-100 µl of ChargeSwitch® Elution Buffer (E5) (or TE Buffer, pH 8.5) to the sample and pipet up and down gently 10 times to resuspend the magnetic beads.

   Important: Do not use water for elution. The DNA will not elute due to the poor buffering capacity of water.
3. Incubate at room temperature for 1 minute.
4. Place the sample in the 96-Well Magnetic Separator for 1 minute or until the beads have formed a tight pellet.
5. Without removing the plate from the 96-Well Magnetic Separator, carefully remove the supernatant **containing the DNA** to a fresh, 96 x 300 µl U-bottomed microtiter plate. Take care not to disturb the pellet of beads by angling the pipette such that the tip is pointed away from the pellet (see figure on page 14).
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream applications.

Quantitating DNA Yield

To quantitate yield of your plasmid DNA, we recommend using one of the Quant-iT™ DNA Assay Kits available from Invitrogen (see page vii for ordering information). The Quant-iT™ kits contain a state-of-the-art quantitation reagent, pre-diluted standards, and a pre-made buffer to allow sensitive and accurate fluorescence-based quantitation of dsDNA. For more information about the Quant-iT™ DNA Assay Kits, see www.invitrogen.com or call Technical Service (page 32).

General Information – Automated Sample Processing

Introduction

This section provides general information to use the ChargeSwitch® NoSpin Plasmid Micro Kit (Catalog no. CS10201-10) to process large numbers of samples in 96-well format using an automated liquid handling robot. If you wish to process small numbers of samples individually, see **General Information – Individual Samples**, page 6.

Hardware Requirements

The ChargeSwitch® chemistry is ideal for purification of plasmid DNA using liquid handling robots, avoiding the need for centrifugation steps or the use of ethanol or chaotropic salts. You will need to have the following hardware to perform automated processing of bacterial samples using the ChargeSwitch® NoSpin Plasmid Micro Kit:

- Any liquid handling robotic workstation with a gripper arm
- Appropriate tips for liquid dispensing and aspiration (see below for factors to consider)
- 96-well Magnetic Separator (see page 7)
- Shaker
- 96 x 2 ml deep well plate(s) (Greiner, Catalog no. 780270 or Abgene, Catalog no. AB-0932)
- 96 x 1 ml deep well plates (Greiner, Catalog no. 780201)
- 96 x 300 µl U-Bottomed microtiter plate (Greiner, Catalog no. 650201)

Tip Selection

You may use any tips of choice to dispense and aspirate liquid during the purification procedure. Consider the following factors when choosing an appropriate tip to use.

- Fixed vs. disposable tips
- Tip size vs. head size
- Conductive or non-conductive
- Sterile or non-sterile
- Filtered or non-filtered

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General Information – Automated Sample Processing, continued

Primary Liquid Handling Parameters

The table below lists the primary liquid handling parameters required to isolate DNA using the automated protocol. Use the parameters and guidelines provided, as well as the protocol on pages 26-27 to program your robot.

Parameter	Aim	Guidelines
[Magnetic Bead Preparation]	To resuspend beads prior to mixing with solution	• Only required once • Beads stay in suspension for up to 45 minutes
[Mixing #1]	Used to mix beads or bead/DNA pellet with buffer	• Aspirate/dispense at 400-500 µl • Aspirate/dispense position fixed 1-2 mm above well bottom • Use tips/volume setting at 80 µl volume
[Dispense liquid]	Normal liquid parameters for adding a reagent to each well	• Aspirate/dispense at 300-400 µl • Use multi-dispense if appropriate to save time
[Transfer supernatant to waste]	To remove and discard supernatant	• Aspirate slowly at 50-100 µl/second • Aspirate off the entire liquid volume using liquid detect and tracking or setting fixed height 1 mm above well bottom • Do not disturb pellet • Dispense to waste
[Transfer supernatant to another plate]	To transfer supernatant to another plate	• Aspirate slowly at 50-100 µl/second • Aspirate off the entire liquid volume using liquid detect and tracking or setting fixed height 1 mm above well bottom • Do not disturb pellet • Dispense slowly at 50-100 µl/second • Avoid splashing
[Final DNA Elution]	To dispense the eluate containing DNA	• Dispense at 10 µl/second • Aspirate from position fixed 1 mm above well bottom • Avoid bead carry-over • Dispense into new plate at 2 mm above well bottom

Automated Plasmid DNA Isolation

Introduction

This section provides a general protocol for automated isolation of plasmid DNA from 1 ml bacterial samples in a 96-well format using the ChargeSwitch® NoSpin Plasmid Micro Kit (Catalog no. 10201-10). Use this general protocol to develop the script for your liquid handling robot.

Ambient Temperature

Perform the purification procedure at ambient (room) temperature. Make sure that the temperature is between 17-23°C. Alkaline lysis is adversely affected when the ambient temperature is > 23°C, resulting in lower plasmid DNA yield.

Important

Two types of magnetic beads are provided in each kit:

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads

These beads are not interchangeable. Do not substitute one type of beads for the other at each appropriate specified point in the purification procedure.

Materials Needed

Have the following materials on hand before beginning:

- Liquid handling robot configured to process samples in 96-well plates
- 1 ml bacterial culture samples
- 96 x 2 ml deep well plates
- 96 x 300 µl U-bottomed microtiter plates

Components Supplied with the Kit

- ChargeSwitch® MagnaClear™ Beads
- ChargeSwitch® Magnetic Beads
- RNase A
- ChargeSwitch® Resuspension Buffer (R4)
- ChargeSwitch® Lysis Buffer (L9)
- ChargeSwitch® Precipitation Buffer (N5)
- ChargeSwitch® Wash Buffer (W11)
- ChargeSwitch® Wash Buffer (W12)
- ChargeSwitch® Elution Buffer (E5) or TE Buffer (not supplied; 10 mM Tris-HCl, 1 mM EDTA, pH 8.5)

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Automated Plasmid DNA Isolation, continued

Important Guidelines

To maximize DNA yield, follow these recommendations when processing your samples:

- Ensure that the robotic tips enter the wells of the plates without interfering with the pellet of beads.
- When removing supernatant, aspirate slowly to ensure that the pellet of beads is not disturbed.
- When removing supernatant after binding the bacterial cells to the MagnaClear™ Beads (before lysis), leave some residual liquid behind to ensure the pellet of beads is not disturbed. Leaving supernatant behind at this stage will not affect the results.
- When resuspending pelleted ChargeSwitch® MagnaClear™ Beads or ChargeSwitch® Magnetic Beads, make sure that all beads are fully resuspended to maximize DNA recovery.
- When shaking the plate after adding ChargeSwitch™ Precipitation Buffer (N5), shake only until the precipitate is broken up into small fragments. Do not shake excessively as this shears genomic DNA, and may result in co-purification of some genomic DNA.
- To maximize DNA yield, make sure that all Wash Buffer is removed before elution.
- To maximize DNA yield, make sure that the beads are fully resuspended during the elution step.

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Automated Plasmid DNA Isolation, continued

Before Starting

Perform the following before beginning:

ChargeSwitch® Resuspension Buffer (R4)

Add the entire contents of the supplied RNase A (2.5 ml) to the bottle of ChargeSwitch® Resuspension Buffer (R4; 125 ml). Mix well (total volume = 127.5 ml). Mark the tube label to indicate that RNase A is added. Store the buffer with RNase A at 4°C.

ChargeSwitch® Precipitation Buffer (N5)

Chill the Precipitation Buffer (N5) at 4°C.

ChargeSwitch® Lysis Buffer (L9)

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

ChargeSwitch® MagnaClear™ Beads and ChargeSwitch® Magnetic Beads

Vortex each tube of magnetic beads to fully resuspend and evenly distribute the beads in the storage buffer before use.

Automated Protocol

Follow the protocol below to isolate plasmid DNA from 1 ml bacterial cultures. The volumes given are on a per sample basis.

1. Start with 96 x 1 ml bacterial cultures in a 96 x 2 ml deep well plate.
2. Add 30 µl of ChargeSwitch® MagnaClear™ Beads.
3. Shake at medium speed for 1 minute at room temperature.
4. Move samples to the 96-Well Magnetic Separator.
5. Wait for 1 minute or until the beads have formed a tight pellet.
6. Slowly aspirate the supernatant and discard, leaving behind the pellet of beads. Some residual liquid remaining is acceptable.
7. Move samples to the shaker.
8. Add 100 µl of Resuspension Buffer (R4) containing RNase A.

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Automated Plasmid DNA Isolation, continued

Automated Protocol, continued

9. Shake at top speed until the pellet of beads is fully resuspended in solution. This make take several minutes.
10. Add 100 µl of Lysis Buffer (L9).
11. Shake at medium speed for 1 minute.
12. Wait for 1 minute.
13. Add 100 µl of ChargeSwitch® Precipitation Buffer (N5).
14. Shake at medium fast speed for 1 minute, then at medium speed for 1 minute.

    Note: The shaking step breaks up the precipitate into smaller fragments that can be easily separated on the magnet. Do not shake excessively as this shears genomic DNA, resulting in co-purification of some genomic DNA.
15. Move samples to the 96-Well Magnetic Separator.
16. Wait for 1 minute or until the beads have formed a tight pellet.
17. Remove the supernatant (~ 220 µl) to a 96 x 1 ml deep well plate. Discard the first plate.
18. Move the samples to the 96-Well Magnetic Separator.
19. Wait for 1 minute or until the beads have formed a tight pellet. During this time, dispense 20 µl of ChargeSwitch® Magnetic Beads (make sure beads are fully resuspended before addition) into a second 96 x 1 ml deep well plate.
20. Remove the supernatant (~200 µl) to the 96 x 1 ml deep well plate containing the ChargeSwitch® Magnetic Beads.
21. Shake at slow to medium speed for 1 minute at room temperature to mix.
22. Move the samples to the 96-Well Magnetic Separator.
23. Wait for 1 minute.
24. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
25. Move samples to the shaker.
26. Add 200 µl of ChargeSwitch® Wash Buffer (W11).

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Automated Plasmid DNA Isolation, continued

Automated Protocol, continued

27. Shake at medium speed for 1 minute at room temperature.
28. Move the samples to the 96-Well Magnetic Separator.
29. Wait for 1 minute.
30. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
31. Move samples to the shaker.
32. Add 200 µl of ChargeSwitch® Wash Buffer (W12).
33. Shake at medium speed for 1 minute at room temperature.
34. Move the samples to the 96-Well Magnetic Separator.
35. Wait for 1 minute.
36. Slowly aspirate all of the supernatant and discard, leaving behind the pellet of beads.
37. Move samples to the shaker.
38. Add 100 µl of ChargeSwitch® Elution Buffer (E5).

    Note: You may vary elution volume depending on your needs. Do not elute in volumes < 60 µl as the DNA may not completely elute from the beads.
39. Shake at top speed for 1 minute to fully resuspend the beads.
40. Wait for 1 minute.
41. Move the samples to the 96-Well Magnetic Separator.
42. Wait for 1 minute.
43. Slowly aspirate the **supernatant containing the DNA** to a 96 x 300 µl U-bottomed microtiter plate.

Storing DNA

Store the purified DNA at -20°C or use immediately for downstream applications.

Quantitating DNA Yield

To quantitate yield of the plasmid DNA, use one of the Quant-iT™ DNA Assay Kits. See page 15 for more information.

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	• Use fresh overnight culture with an OD_{600} up to 6. • Grow *E. coli* in LB Medium or a richer medium such as Terrific Broth.
	Incomplete lysis	• Use chilled Precipitation Buffer (N5). • After adding Precipitation Buffer (N5), shake at top speed until a fine, grainy, brown precipitate has formed. • Increase the incubation time during lysis. Shake at medium speed.
	Insufficient amount of ChargeSwitch® MagnaClear™ Beads added	Vortex the tube containing the ChargeSwitch® MagnaClear™ Beads to fully resuspend the beads in solution before use.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in solution before use.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ or 96-well Magnetic Separator when removing supernatant during the binding or washing steps. • Remove the supernatant without disturbing the pellet of beads by angling the pipette tip away from the pellet.

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Troubleshooting, continued

Problem	Cause	Solution
Low DNA yield, continued	Bubbles formed during mixing steps	Make sure that the pipette tip is submerged in the solution during mixing.
	Incomplete dissociation of DNA from the ChargeSwitch® Magnetic Beads	Perform additional mixing of the suspension of beads (by pipetting up and down).
	Ambient temperature > 23°C	Perform purification at ambient temperatures between 17-23°C. Performing the procedure at ambient temperature > 23°C can adversely affect alkaline lysis.
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer (E5) to the sample, pipet up and down to fully resuspend the magnetic beads before incubation. • Do not use water to elute DNA. Use ChargeSwitch® Elution Buffer (E5) or TE, pH 8.5.
	Lysate mixed too vigorously or small pipette tips used during mixing	• Use the appropriate pipette tip set to a volume lower than the total volume of solution in the sample. • Pipet up and down gently to mix.
No DNA recovered	Water used for elution	Do not use water for elution. The elution buffer **must** have a pH = 8.5-9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads. Use Elution Buffer (E5) or TE, pH 8.5.
	Used ChargeSwitch® Magnetic Beads during lysate preparation step	You must use ChargeSwitch® MagnaClear™ Beads during lysate preparation step.

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Troubleshooting, continued

Problem	Cause	Solution
No DNA recovered, continued	ChargeSwitch® Magnetic Beads stored or handled improperly	• Store beads at room temperature. Do not freeze the beads as they will become irreparably damaged. • Make sure that the beads are in solution at all times and do not become dried. Dried beads are non-functional.
	Purification Mix did not contain ChargeSwitch® Magnetic Beads	Purification Mix should contain ChargeSwitch® Purification Buffer (N6) + ChargeSwitch® Magnetic Beads.
Bacterial lysate is cloudy	Insufficient amount of ChargeSwitch® MagnaClear™ Beads added	Vortex the tube containing the ChargeSwitch® MagnaClear™ Beads to fully resuspend the beads in solution before use.
	Precipitation Buffer not chilled	Use chilled ChargeSwitch® Precipitation Buffer.
	Sample not sufficiently shaken	After adding chilled ChargeSwitch® Precipitation Buffer, shake at top speed until a fine, grainy, brown precipitate has formed.
DNA not pure (*e.g.* contains RNA)	Used ChargeSwitch® Resuspension Buffer without RNase A	Use ChargeSwitch® Resuspension Buffer containing RNase A during lysate preparation step.
DNA is degraded	DNA contaminated with DNases	Maintain a sterile environment while working (*i.e.* wear gloves and use DNase-free reagents).

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).

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, CA 92008 USA
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 602 6500
E-mail:
tech_service@invitrogen.com

European Headquarters:
Invitrogen Ltd
Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF, UK
Tel: +44 (0) 141 814 6100
Tech Fax: +44 (0) 141 814 6117
E-mail:
eurotech@invitrogen.com

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Technical Service, continued

MSDS Requests

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Purchaser Notification and Product Qualification

Purchaser Notification

Limited Use Label License No. 265: ChargeSwitch® Technology

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

Product 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.

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA
Phone +1 760 603 7200 | Toll Free in USA 800 955 6288

For support visit
www.lifetechnologies.com/support or email techsupport@lifetech.com

www.lifetechnologies.com