Invitrogen™
life technologies

Instruction Manual

ChargeSwitch® gDNA 1 ml Serum Kit

For purification of genomic DNA from 0.2-1 ml samples of human serum

Catalog no. CS11040

Version A

3 January 2005

25-0834

Table of Contents

Kit Contents and Storage

Shipping and Storage

All components of the ChargeSwitch® gDNA 1 ml Serum Kit are shipped at room temperature. Upon receipt, store the Proteinase K at 4°C. Store all other components at room temperature.

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

Contents

The components supplied in the ChargeSwitch® gDNA 1 ml Serum Kit are listed below. The reagents supplied are sufficient to perform 50 purifications from 1 ml serum samples.

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

Component	Amount
ChargeSwitch® Lysis Buffer (L19)	50 ml
ChargeSwitch ® Magnetic Beads	1.5 ml
Proteinase K (20 mg/ml in 50 mM Tris-HCl, pH 8.5, 5 mM $CaCl_2$, 50% glycerol)	1.5 ml
ChargeSwitch® Purification Buffer (N7)	20 ml
ChargeSwitch® Wash Buffer (W12)	100 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	10 ml

Accessory Products

Additional Products

The table below lists additional products available from Invitrogen that may be used with the ChargeSwitch® gDNA 1 ml Serum Kit. In addition, the table lists a selection of ChargeSwitch® gDNA Kits that are available for purification of genomic DNA from other sources. For more information about these and other ChargeSwitch® gDNA Kits, refer to our Web site at www.invitrogen.com or call Technical Service (see page 15).

Product	Amount	Catalog no.
MagnaRack™	1 rack	CS15000
ChargeSwitch® gDNA 20 µl Blood Kit	96 purifications	CS11010
ChargeSwitch® gDNA 100 µl Blood Kit	50 purifications	CS11000
ChargeSwitch® gDNA 1 ml Blood Kit	20 purifications	CS11001
ChargeSwitch® gDNA 50 µl Sheep Blood Kit	50 purifications	CS11300
ChargeSwitch® gDNA Micro Tissue Kit	50 purifications	CS11203
ChargeSwitch® gDNA Mini Tissue Kit	25 purifications	CS11204
ChargeSwitch® gDNA Mini Bacteria Kit	50 purifications	CS11301
ChargeSwitch® gDNA Normalized Buccal Cell Kit	50 purifications	CS11020
ChargeSwitch® gDNA Buccal Cell Kit	50 purifications	CS11021
ChargeSwitch® Forensic DNA Purification Kit	100 purifications	CS11200
Quant-iT™ PicoGreen® dsDNA Quantitation Kit	200-2000 assays	P7589
Quant-iT™ PicoGreen® dsDNA Quantitation Reagent	200-2000 assays (1 ml)	P7581

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 percentages and well formats. In addition, a large variety of DNA ladders are available from Invitrogen for sizing DNA. For more information about these products, see www.invitrogen.com or call Technical Service.

Introduction

Overview

Introduction

The ChargeSwitch® gDNA 1 ml Serum Kit allows rapid and efficient purification of genomic DNA from 0.2-1 ml samples of human serum. After preparing the lysates, you may purify DNA in less than 15 minutes using the ChargeSwitch® Technology. For more information about the ChargeSwitch® Technology, see page 2.

Intended Use for the Kit

The ChargeSwitch® gDNA 1 ml Serum Kit is designed to allow isolation of up to 200 ng of genomic DNA from 0.2-1 ml samples of fresh or frozen human serum with or without EDTA. The purified DNA is suitable for use in downstream applications including PCR and qRT-PCR.

Note: DNA yield will vary from 1-200 ng according to sample source and storage method. The average DNA yield is 30 ng/ml of serum from healthy individuals.

Advantages

Use of the ChargeSwitch® gDNA 1 ml Serum Kit to isolate genomic DNA provides the following advantages:

- Uses a magnetic bead-based technology to isolate genomic DNA without the need for hazardous chemicals, centrifugation, or vacuum manifolds
- Rapid and efficient purification of genomic DNA from 0.2-1 ml of human serum in less than 15 minutes following sample preparation and lysis
- Simple lysis of serum components with Proteinase K without the need for any mechanical lysis
- Minimal contamination with RNA
- The purified genomic DNA demonstrates improved downstream performance in applications including PCR and qRT-PCR

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Overview, continued

System Specifications

Starting Material:	0.2-1 ml of fresh or frozen human serum, with or without EDTA
Elution Volume:	50 µl
DNA Yield:	1-200 ng
DNA Size:	50 bp-1.5 kb

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.

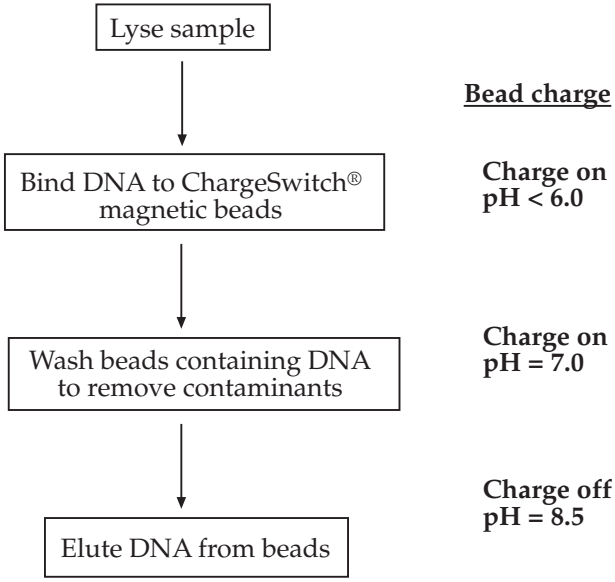
ChargeSwitch® Magnetic Bead Specifications

Bead Binding Capacity:	5-10 µg genomic 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

Experimental Outline

Introduction

The figure below illustrates the basic steps necessary to purify genomic DNA from human serum using the ChargeSwitch® gDNA 1 ml Serum Kit.



Methods

General Information

User Supplied Materials

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

- A magnetic separation rack suitable for use with 1.5 ml microcentrifuge tubes (see below)
- Sterile, 1.5 ml microcentrifuge tubes
- RNase A (optional; 5 mg/ml in 10 mM Tris-HCl, pH 8.5, 10 mM EDTA)
- Vortex mixer
- 20 µl, 200 µl, and 1 ml sterile, pipette tips

MagnaRack™

The MagnaRack™ 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 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 15).

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General Information, continued

Safety Information

Follow the safety guidelines below when using the ChargeSwitch® gDNA 1 ml Serum Kit.

- 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 Beads

Follow the guidelines below when handling the ChargeSwitch® Magnetic 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, 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 genomic DNA in 50 µl of ChargeSwitch® Elution Buffer (E5). You may vary the amount of ChargeSwitch® Elution Buffer (E5) used to obtain genomic 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 Genomic DNA

Introduction

This section provides guidelines and instructions to isolate genomic DNA from 0.2-1 ml samples of human serum.

Starting Material

Use this procedure to isolate genomic DNA from 0.2-1 ml samples of fresh or frozen human serum, with or without EDTA.

Materials Needed

Have the following materials on hand before beginning:

- Serum sample(s) (see above)
- MagnaRack™ (Catalog no. CS15000) or other magnetic separation rack
- 5 mg/ml RNase A (optional)
- Sterile 1.5 ml microcentrifuge tubes
- Vortex mixer
- Sterile pipette tips (20 µl, 200 µl, and 1 ml)

Components Supplied with the Kit

- ChargeSwitch® Lysis Buffer (L19)
- Proteinase K
- ChargeSwitch® Magnetic Beads
- ChargeSwitch® Purification Buffer (N7)
- 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 Genomic DNA, continued

Suggested Reagent Volumes

Use the protocol in this manual to isolate genomic DNA from 1 ml samples of human serum. To isolate DNA from smaller sample volumes (*i.e.* 0.2-0.8 ml of human serum), scale down the volume of reagents used according to the table below.

	Serum Volume				
Reagent	**1 ml**	**800 µl**	**600 µl**	**400 µl**	**200 µl**
ChargeSwitch® Lysis Buffer (L19)	700 µl	560 µl	420 µl	280 µl	140 µl
Proteinase K	30 µl	30 µl	30 µl	30 µl	30 µl
RNase A (optional)	5 µl	5 µl	5 µl	5 µl	5 µl
ChargeSwitch® Purification Buffer (N7)	250 µl	200 µl	150 µl	100 µl	50 µl
ChargeSwitch® Magnetic Beads	30 µl	24 µl	18 µl	12 µl	6 µl
ChargeSwitch® Wash Buffer (W12)	1 ml	800 µl	600 µl	400 µl	200 µl
ChargeSwitch® Elution Buffer (E5)	50 µl	50 µl	50 µl	50 µl	50 µl

Before Starting

Before starting, prepare **Lysis Mix** as follows. For each 1 ml serum sample, mix 700 µl of ChargeSwitch® Lysis Buffer (L19) and 30 µl of Proteinase K to prepare the Lysis Mix. When isolating DNA from multiple samples, scale up the volume of reagents used and prepare a master Lysis Mix.

Preparing the Lysate

Follow the procedure below to prepare a lysate from a 1 ml serum sample. For smaller sample volumes, use the reagent amounts specified in the table above.

1. Place the 1 ml serum sample in a sterile microcentrifuge tube. Add 730 µl of Lysis Mix (see above).
2. *Optional:* Add 5 µl of RNase A.
3. Pipet up and down 5 times to mix.

   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. For smaller sample volumes, use an appropriate size pipette tip to ensure thorough mixing while avoiding bubble formation.
4. Incubate at room temperature for 20 minutes to lyse the sample. Alternatively, incubate on a rotating mixer.
5. Proceed to **Binding DNA**, next page.

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Isolating Genomic DNA, continued

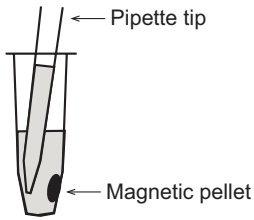
Binding DNA

Follow the procedure below to bind the 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. Make sure that all of the solution containing beads is at the bottom of the tube.
2. Add 250 µl of ChargeSwitch® Purification Buffer (N7) to the digested sample (from Step 4, previous page).

   Note: Adding the ChargeSwitch® Purification Buffer (N7) lowers the pH of the sample, and optimizes the binding conditions.
3. Add 30 µl of ChargeSwitch® Magnetic Beads (from Step 1) and pipet up and down gently 5 times to mix.

   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.
4. Incubate at room temperature for 2 minutes to allow the DNA to bind to the ChargeSwitch® Magnetic Beads.
5. Place the sample in the MagnaRack™ for 3 minutes or until the beads have formed a tight pellet.
6. 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).



7. Proceed immediately to **Washing DNA**, next page.

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Isolating Genomic DNA, continued

Washing DNA

1. Remove the tube containing the pelleted magnetic beads from the MagnaRack™ (Step 6, previous page). There should be no supernatant in the tube.
2. Add 1 ml of ChargeSwitch® Wash Buffer (W12) to the tube and pipet up and down gently 5 times 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 2 minutes 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 9).
5. Repeat Steps 1-4.
6. Proceed to **Eluting DNA**, next page.

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Isolating Genomic DNA, 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 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 2 minutes.
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 9).

   Note: If the eluate containing the DNA is discolored, repeat Steps 4-5.
6. Discard the used magnetic beads. Do not reuse the beads.

Storing DNA

Store the purified DNA at -20°C or use immediately for PCR, qRT-PCR, or other appropriate downstream application. Avoid repeatedly freezing and thawing DNA.

Quantitating DNA Yield

The yield of genomic DNA obtained from 1 ml (or less) of human serum is insufficient to allow quantitation by measuring UV absorbance at 260 nm. To quantitate yield and verify the quality of your DNA, we recommend using the Quant-iT™ PicoGreen® dsDNA Quantitation Kit (Catalog no. P7589) available from Invitrogen. This kit contains the reagents necessary to allow sensitive and accurate fluorescence detection of as little as 25 pg/ml of dsDNA using the Quant-iT™ PicoGreen® dsDNA Quantitation Reagent. Alternatively, you may perform Alu repeat analysis using qRT-PCR.

Troubleshooting

Introduction

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

Problem	Cause	Solution
Low DNA yield	Incomplete lysis	• Be sure to add Proteinase K during lysis. • Increase the length of incubation at room temperature. • Perform incubation on a rotating mixer.
	Insufficient amount of ChargeSwitch® Magnetic Beads added	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads in solution before adding them to your sample.
	Pellet of beads disturbed or lost during binding or washing steps	• Keep the sample in the MagnaRack™ 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.
	Incomplete dissociation of DNA from the ChargeSwitch® Magnetic Beads	Perform additional mixing of the suspension of beads (by pipetting up and down).
	Incorrect elution conditions	• After adding ChargeSwitch® Elution Buffer (E5) to the sample, pipet up and down to resuspend the magnetic beads before incubation. • Do not use water to elute DNA. Use Elution Buffer (E5) or TE, pH 8.5.

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Troubleshooting, continued

Problem	Cause	Solution
No DNA recovered	Lysis Mix added to sample instead of ChargeSwitch® Purification Buffer (N5)	You must add ChargeSwitch® Purification Buffer (N5) to your sample (in the presence of magnetic beads) to lower the pH, allowing DNA to bind to the beads.
	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 ChargeSwitch® Elution Buffer (E5) or TE, pH 8.5-9.0.
	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. Beads that have dried out are non-functional.
DNA contains contaminants	Beads not fully resuspended during washing steps	Thoroughly resuspend the pellet of magnetic beads by pipetting up and down.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	Repeat the elution step (**Eluting DNA**, Steps 4-5, page 11).

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Troubleshooting, continued

Problem	Cause	Solution
DNA is sheared or degraded	Lysate mixed too vigorously or small pipette tips used during mixing	• Use a 1 ml pipette tip set to 900 µl to mix the sample. • Pipet up and down gently to mix.
	Bubbles formed during mixing steps	Make sure that the pipette tip is submerged in the solution during mixing.
	DNA repeatedly frozen and thawed	Aliquot DNA and store at 4°C or -20°C. Avoid repeated freezing and thawing.
	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

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.

Limited Warranty

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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.

Notes

Notes

Notes

Corporate Headquarters:
Invitrogen Corporation
1600 Faraday Avenue
Carlsbad, California 92008
Tel: 1 760 603 7200
Tel (Toll Free): 1 800 955 6288
Fax: 1 760 603 7229
Email: tech_service@invitrogen.com

European Headquarters:
Invitrogen Ltd
3 Fountain Drive
Inchinnan Business Park
Paisley PA4 9RF, UK
Tel (Free Phone Orders): 0800 269 210
Tel (General Enquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287
Email: eurotech@invitrogen.com

International Offices:
Argentina 5411 4556 0844
Australia 1 800 331 627
Austria 0800 20 1087
Belgium 0800 14894
Brazil 0800 11 0575
Canada 800 263 6236
China 10 6849 2578
Denmark 80 30 17 40

France 0800 23 20 79
Germany 0800 083 0902
Hong Kong 2407 8450
India 11 577 3282
Italy 02 98 22 201
Japan 03 3663 7974
The Netherlands 0800 099 3310
New Zealand 0800 600 200
Norway 00800 5456 5456

Spain & Portugal 900 181 461
Sweden 020 26 34 52
Switzerland 0800 848 800
Taiwan 2 2651 6156
UK 0800 838 380
For other countries see our Web site

www.invitrogen.com