USER GUIDE

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ChargeSwitch® gDNA Rendered Meat Purification Kit

Purification of genomic DNA (gDNA) from cattle feed, meal, and heparin products

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For testing of Food and Environmental samples only.

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About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description
A.0	December 2014	• Added heparin protocol. • Updated protocol organization to align with current style. • Updated user guide template with associated updates to covers, legal, document support, and safety sections. • New document control nomenclature.
1.0	2006	New document

Product information

Product description

The ChargeSwitch® gDNA Rendered Meat Purification Kit allows rapid and efficient purification of PCR-ready genomic DNA (gDNA) from:

- Animal feed (500 mg)
- Animal meal (250 mg)
- Heparin (200–250 mg)

The kit uses magnetic bead-based technology for purification of gDNA without centrifugation, vacuum manifolds, or organic solvents. Genomic DNA can be prepared from sample lysates in less than 15 minutes, when processing 1–5 samples. The purified DNA has minimal RNA contamination, and it is suitable for analysis using real-time quantitative PCR (qPCR) or another method of choice, for identification of mammalian DNA in samples.

ChargeSwitch® technology is a magnetic bead-based technology that utilizes a switchable surface whose charge is dependent on the pH of the surrounding buffer (Figure 1).

- In low pH conditions, the ChargeSwitch® Magnetic Beads have a positive charge that binds the negatively charged nucleic acid backbone. Proteins and other contaminants remain unbound and are removed in an aqueous wash buffer.
- To elute the nucleic acid, the charge on the surface of the ChargeSwitch® Magnetic Beads is neutralized by raising the pH to >8.5 using a low salt elution buffer. Purified DNA elutes instantly into the elution buffer and is ready for use in downstream applications.

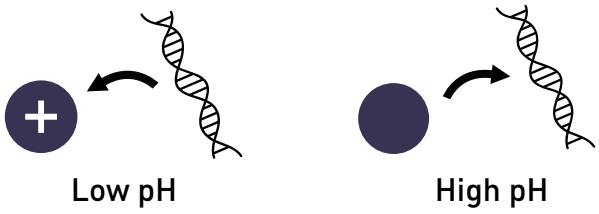


Figure 1 ChargeSwitch® technology

Kit contents and storage

Component	Amount[1]	Storage[2]
ChargeSwitch® Magnetic Beads (25 mg/ml in 10 mM MES, pH 5.0, 10 mM NaCl, 0.1% Tween 20)	1.1 mL	Room temperature
ChargeSwitch® SDS	10 mL	
ChargeSwitch® 10% Detergent	10 mL	
ChargeSwitch® Lysis Buffer	100 mL	
ChargeSwitch® Precipitation Buffer (N5)	38.5 mL	
ChargeSwitch® Wash Buffer (W12)	200 mL	
ChargeSwitch® Elution Buffer (E5; 10 mM Tris HCl, pH 8.5)	15 mL	

[1] 50 purifications from feed or meal samples; 25 purifications from heparin samples.
[2] All components are guaranteed stable for 6 months when stored properly.

Required materials not provided with the kit

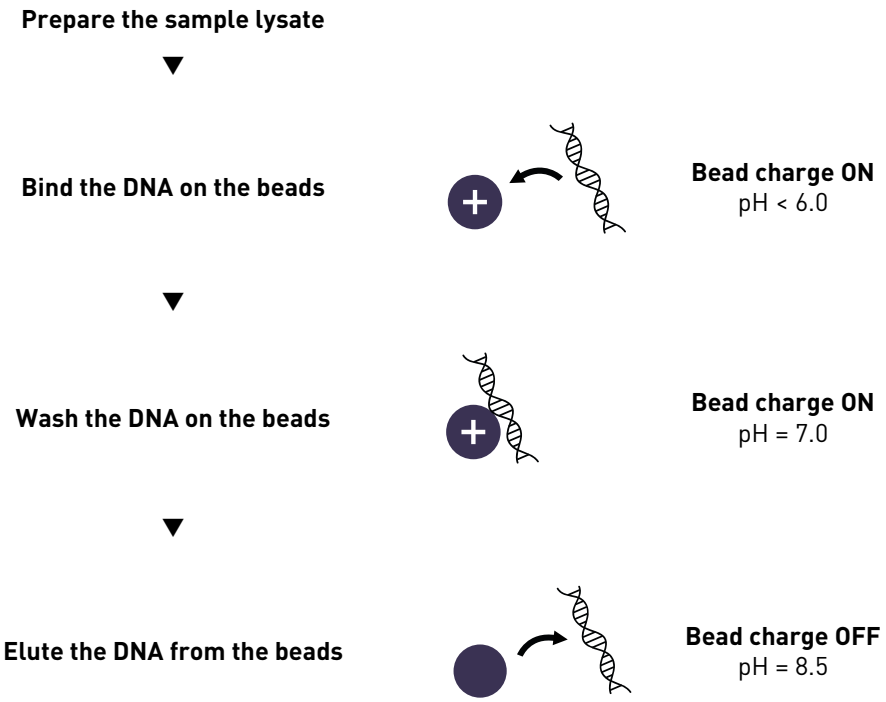
Unless otherwise indicated, all materials are available from Life Technologies (**www.lifetechnologies.com**). MLS: Fisher Scientific (**www.fisherscientific.com**) or other major laboratory supplier.

Product	Catalog number
MagnaRack™ Magnetic Rack	CS15000
For animal feed samples: No. 6 wire-mesh sieve (3.35 mm opening)	MLS
Sterile 2.0-mL microcentrifuge tubes, with locking lids **Note:** Locking lids are recommended to prevent the lid from popping open during the 95°C incubation.	MLS
Adjustable pipettes and aerosol barrier pipette tips	MLS
1.5-mL microcentrifuge tubes	MLS
Microcentrifuge	MLS
Water bath or heat block set to 95°C	MLS
Smart Spatula	MLS
Materials for DNA quantification using one of these methods:	
UV absorbance: spectrophotometer and accessories	MLS

Product	Catalog number
Fluorescence technology: Quant-iT™ DNA assay kits	—
• Quant-iT™ DNA Assay Kit, High Sensitivity	Q33120
• Quant-iT™ DNA Assay Kit, Broadrange	Q33130
• Quant-iT™ PicoGreen® dsDNA Assay	P7589

Methods

Workflow



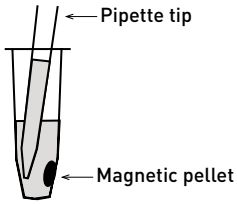
Important procedural guidelines

Handling magnetic beads

For best results:

- Do not freeze ChargeSwitch® Magnetic Beads—freezing damages the magnetic property of the beads. Store the beads at room temperature.
- Always keep the ChargeSwitch® Magnetic Beads in solution. Do not allow the beads to dry, including during washing procedures, as this renders the beads non-functional.
- Vortex the ChargeSwitch® Magnetic Beads to resuspend thoroughly before pipetting.
- During mixing steps, to avoid forming bubbles:
  - Use an adjustable pipette set to a specific volume as directed in the protocol.
  - Pipet up and down gently with the pipette tip submerged in the solution.

- To aspirate the supernatant after bead washing, point the pipette tip away from the beads, and carefully remove the supernatant without disturbing the beads.



- Discard ChargeSwitch® Magnetic Beads after use.

Sample processing

To maximize DNA yield:

- Maintain a sterile environment when handling DNA to avoid any contamination from DNases.
- Ensure that no DNases are introduced into the solutions supplied with the kit.
- Make sure all equipment that contacts DNA is sterile, including pipette tips and tubes.
- Perform all steps at room temperature, unless otherwise noted.
- Make sure that:
  - The ChargeSwitch® Wash Buffer is removed completely before elution.
  - The ChargeSwitch® Magnetic Beads are fully resuspended during the elution step.

Prepare the sample lysate

1. Transfer the indicated amount of sample to sterile 2.0-mL microcentrifuge tubes.

Sample type	Amount
Animal feed	500 mg; transfer to two tubes. **Note:** Sift feed with a No. 6 wire-mesh sieve or equivalent before weighing sample, to remove large components of the feed (for example, whole corn kernels and whole grains) that can reduce the sensitivity of downstream assays.
Animal meal	250 mg
Heparin	200–250 mg

2. At room temperature, add 1 mL of ChargeSwitch® Lysis Buffer (L15) and 100 µL of ChargeSwitch® SDS to each tube.

3. Mix the sample with a ChargeSwitch® Lysis Buffer and ChargeSwitch® SDS either with Smart Spatula or by inversion

For these samples...	To mix...
Heparin, some feed and meal samples	Use a Smart Spatula, then lock the tube lid.
All other samples	Lock the tube lid, invert five times, then gently tap the tube on a hard surface to remove sample that is stuck to the lid.

4. Incubate in a 95°C water bath or heat block for 5 minutes.

CAUTION! Be careful when opening the tubes after heating.

5. Carefully open each tube, add 400 µL of ChargeSwitch® Precipitation Buffer (N5) to the lysate, then mix by inversion.

6. Place each tube on ice for 5 minutes to precipitate the proteins.

7. Centrifuge the tube at 17,000 × *g* for 5 minutes at room temperature to pellet the debris.

   Note: The supernatant contains the lysate.

Bind the DNA to the beads

1. Carefully transfer the indicated volume of lysate to a sterile 1.5-mL microcentrifuge tube.

Sample type	Volume
Animal feed	500 µL from each tube of lysate for a total of ~1000 µL
Animal meal	500 µL
Heparin	1200 µL **Note:** It may not be possible to recover 1200 µL of lysate, due to differences in heparin products.

2. Add 100 µL of ChargeSwitch® 10% Detergent (D1) to the tube of lysate.

3. Thoroughly vortex the tube of ChargeSwitch® Magnetic Beads, and add the indicated volume of beads to the sample.

Sample type	Volume
Animal feed	20 µL
Animal meal	20 µL
Heparin	40 µL

4. Mix gently, without forming bubbles, by pipetting up and down 5 times using a 1-mL pipette set to 900 µL.

   IMPORTANT! Avoid forming bubbles by ensuring that the pipette tip is fully submerged during mixing, and by pipetting up and down gently.

5. Incubate the tube at room temperature for 1 minute.

6. Place the tube on the MagnaRack™ Magnetic Rack until the beads have formed a tight pellet and the supernatant has cleared (~ 1 minute).

7. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the bead pellet.

   Note: The lysate is viscous; angle the pipette tip so that it is pointed away from the pellet, and aspirate slowly, to avoid disturbing the bead pellet.

Wash the DNA on the beads

1. Remove the tube from the MagnaRack™ magnet, add 1 mL of ChargeSwitch® Wash Buffer (W12) to the tube, and pipet up and down 5 times using a 1-mL pipette set to 900 µL.

   Note: Pipet up and down to gently mix without forming bubbles.

2. Place the tube on the magnet for ~1 minute until the beads have formed a tight pellet and the supernatant is clear.

3. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the bead pellet.

   Note: Heparin samples are very viscous; pipet slowly to ensure that beads are not removed from the magnet. Removal of beads will decrease the DNA yield.

4. Remove the tube from the MagnaRack™ magnet, add 750 µL of ChargeSwitch® Wash Buffer (W12) and mix up and down 5 times.

5. Place the tube on the magnet for ~1 minute until the beads have formed a pellet and the supernatant is clear.

6. Without removing the tube from the magnet, carefully aspirate and discard the supernatant without disturbing the bead pellet.

7. Repeat step 4–step 6 one more time.

   Remove the supernatant as completely as possible after the final wash.

   Note: Keep the pelleted beads, which contain the bound DNA.

Elute the DNA from the beads

1. Remove the tube containing the pelleted magnetic beads from the magnet.

   Note: If any supernatant is visible, carefully remove it before proceeding.

2. Add the indicated amount of ChargeSwitch® Elution Buffer (E5) to the bead pellet.

Sample type	Volume
Animal feed	100 µL
Animal meal	100 µL
Heparin	75 µL

3. Gently pipet up and down gently 10 times using a pipette set to 20 µL less than the volume of buffer used.

4. Incubate at room temperature for 1 minute.

   (*Optional*) For maximum yield, this incubation can be extended up to 5 minutes total, with gentle tip-mixing after 2 minutes.

5. Place the tube on the magnet for ~1 minute until the beads have formed a tight pellet and the supernatant is clear.

   Note: The supernatant contains the purified DNA.

6. Without removing the tube from the magnet, carefully transfer the supernatant containing the DNA to a new, sterile microcentrifuge tube without disturbing the pellet.

7. Discard the used ChargeSwitch® Magnetic Beads.

The purified gDNA is ready for use.

STOPPING POINT (*Optional*) Store at –20°C. Avoid repeated freezing and thawing of gDNA.

Quantify the DNA concentration

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

- **UV absorbance:**
  a. Prepare a dilution of the DNA solution in 10 mM Tris HCl, pH 8.5, mix well.
  b. 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.
  c. Calculate the concentration of DNA using the formula:
     DNA (µg/mL) = A_{260} × 50 × 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:** These kits provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. Each kit contains a fluorescent quantitation reagent, DNA standards for standard curve, and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescence microtiter plate readers or fluorometers.

A

Troubleshooting

Observation	Possible cause	Recommended action
Low DNA yield	Incomplete lysis	Reduce the amount of starting material.
		Be sure to add ChargeSwitch® SDS during lysis.
	Poor quality of starting material	Use fresh sample and process immediately after collection, or freeze the sample at –80°C or in liquid nitrogen. The yield and quality of DNA isolated depends on the type and age of the starting material.
	Incorrect handling of ChargeSwitch® Magnetic Beads	Vortex the tube containing the ChargeSwitch® Magnetic Beads to fully resuspend the beads before adding them to your sample.
	Bead pellet was disturbed or lost during binding or washing steps	Keep the sample on the MagnaRack™ when removing supernatant during the binding or washing steps.
		Remove the supernatant without disturbing the bead pellet. See "Handling magnetic beads" on page 8.
	Suboptimum elution conditions	After adding ChargeSwitch® Elution Buffer (E5) to the sample, pipet up and down to resuspend the ChargeSwitch® Magnetic Beads before incubation.
		Do not use water to elute DNA. Use ChargeSwitch® Elution Buffer (E5).
		Preheating Elution Buffer (E5) to 55–60°C may improve yield.
No DNA recovered	Water used for elution	The elution buffer must be pH 8.5–9.0 or the DNA will remain bound to the ChargeSwitch® Magnetic Beads. Use ChargeSwitch® Elution Buffer (E5).
	ChargeSwitch® Magnetic Beads were 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 are not dried. Dried beads are non-functional.
Eluate containing DNA is discolored	Magnetic pellet disturbed during elution	Place the sample on the MagnaRack™ until the beads form a tight pellet.
		Remove the eluate to a sterile microcentrifuge tube or sterile microtiter plate, without disturbing the bead pellet.

Observation	Possible cause	Recommended action
DNA is sheared or degraded	Lysate mixed too vigorously	Use an appropriate pipette set to a volume that is lower than the total volume of the solution used 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 the eluted DNA and store at 4°C or –20°C. Avoid repeated freezing and thawing.
	DNA contaminated with DNases	Maintain a sterile environment while working (for example, wear gloves and use DNase-free reagents).

B

Supplemental information

System specifications

Table 1 ChargeSwitch® gDNA Rendered Meat Purification Kit specifications

Feature	Specification
Starting material	• Animal feed (500 mg) • Animal meal (250 mg) • Heparin (200–250 mg)
Bead binding capacity	1 mg bead binds 5–10 µg gDNA
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	• 100 µL (feed, meal) • 75 µL (heparin)
DNA yield	Up to 7 µg

Documentation and support

Customer and technical support

Visit **www.lifetechnologies.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Food Safety support

Website: **www.lifetechnologies.com/foodsafety**

Support email: **foodsafety@lifetech.com**

Phone number in North America: 1-800-500-6855

Phone number outside of North America: Visit **www.lifetechnologies.com/support**, select the link for phone support, and select the appropriate country from the dropdown menu.

Limited product warranty

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