QUICK REFERENCE CARD

ChargeSwitch® Direct 8-well or 96 gDNA Kits

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Introduction

The ChargeSwitch® Direct 8-well and 96 gDNA Kits are a solid phase high-throughput genomic DNA (gDNA) isolation system in an 8-well or 96-well format. The kits allow simultaneous, high-throughput isolation of gDNA from 8 or 96 fresh or frozen samples (1-2 x 10^4 cells, 10 µl whole human blood, or cell-free samples) and are compatible with most automated liquid handling workstations.

The ChargeSwitch® Direct gDNA Kits utilize the ChargeSwitch® Technology that provides a switchable surface charge depending on the pH of the surrounding buffer to facilitate nucleic acid purification. Under low pH conditions, the positive surface charge of the ChargeSwitch® Direct gDNA plate binds the negatively charged nucleic acid backbone. Proteins and other contaminants are not bound and are washed away.

To use the kits, cells and blood samples are lysed in Plate Binding and Lysis Buffer allowing the binding of ~50 ng of the lysate gDNA to each ChargeSwitch® gDNA well. The wells are washed with Wash Buffers to efficiently remove contaminants. Use the purified gDNA bound to the wells for any downstream application such as PCR including multiplex, qPCR, STR analysis, and sequencing. Alternatively, purified gDNA can be eluted.

Kit Contents

The kit contents are listed below. Upon receipt, store all components at room temperature. The plates can be stored for up to 12 months.

Components	CS11205 1 x 96 reactions	CS11206 10 x 96 reactions	CS11209 12 x 8-well strips
ChargeSwitch® Plate Binding and Lysis Buffer (L21)	15 ml	125 ml	15 ml
ChargeSwitch® Wash Buffer (W10)	15 ml	125 ml	15 ml
ChargeSwitch® Wash Buffer (W12)	15 ml	125 ml	15 ml
ChargeSwitch® Elution Buffer (E5; 10 mM Tris-HCl, pH 8.5)	15 ml	125 ml	15 ml
ChargeSwitch® Direct 96 gDNA Plates	1 x 96-well plate	10 x 96-well plates	12 x 8-well strips
Plate Sealers or Cap Strips (8 caps/strip)	1 sealer	10 sealers	24 8-cap strips

Quality Control

The ChargeSwitch® Direct gDNA Kits are functionally qualified by isolating gDNA from blood samples as described in this manual. The bound gDNA is subjected to PCR using specific primers. Agarose gel electrophoresis of the PCR reaction must show specific bands.

General Guidelines

- Wear a laboratory coat, disposable gloves, and eye wear when handling plates and potentially infectious samples such as blood.

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Purification Procedure

General Guidelines, continued

- Make sure all kit reagents are at room temperature before starting.
- Always use proper aseptic techniques when working with DNA and use only sterile, DNase-free tips to prevent DNase contamination.
- If you are using only part of the plate for purification, cover unused wells with the Plate Sealer and leave sealer attached to the plate while purifying gDNA in the other wells. Exposure of unused wells to temperatures of 10 x 30 cycles of PCR reactions does not affect the gDNA binding for future purifications. Store the plates at room temperature for up to 12 months.
- For the ChargeSwitch® 8-well gDNA Kit, two sets of caps are provided. Protect unused ChargeSwitch coated 8-well strips with the plastic rack cover or use a set of caps to cover your unused strips. To ensure the flat caps have a tight seal, additional caps are provided for the PCR.

Preparing Lysates

Prepare your sample lysates as described below. If you have limited sample and wish to perform multiple downstream applications with the purified gDNA, you can reuse the lysates and bind to additional wells (see ***Optional***: **Binding of gDNA to Multiple Wells**).

1. Grow cells in a flask, or in 24-, 48-, or, 96-well cell culture plates.
2. Harvest cells as follows:

   Suspension cells: Harvest suspension cells by centrifugation.

   Adherent cells: Aspirate the medium from culture plate or flask and perform trypsinization of cells. Wash trypsinized cells once with Phosphate Buffered Saline (PBS, cat. no. 10010-023) and aspirate the PBS completely.
3. Resuspend the cells from Step 2 in an appropriate volume of PBS to obtain 1-2 x 10^6 cells/ml. Proceed immediately to **Binding Step**, below.

Binding Step

1. Add 100 µl Plate Binding and Lysis Buffer (L21) to each well as appropriate for the kit you are using.
2. Add your samples to the well as described:

   Cell lysate: Transfer 10 µl of the adjusted cell suspension containing 1-2 x 10^4 cells in PBS from Step 3, above, into each well.

   Human whole blood: Transfer 10 µl human whole blood (fresh, frozen, containing anticoagulants such as EDTA, citrate, heparin, or Alsever's solution) into each well.

   Cell-free samples: Transfer up to 20 µl cell-free sample containing DNA (≥5 µg/ml).
3. Mix well by pipetting up and down 5 times.
4. Incubate for 30 minutes at room temperature to allow binding of gDNA to the plate.

   Note: You may shorten the incubation time to 1 minute, but a shorter incubation time may lead to a reduced yield of gDNA.
5. Proceed to **Washing Step**, next page.

Optional: Binding gDNA to Multiple Wells

If you need to perform multiple downstream applications with samples that are limited, bind the gDNA from your sample lysates to multiple wells.

1. Prepare sample lysates and bind the lysate gDNA to the wells of the first plate or strip for your specific sample type as described on the previous page.
2. Transfer the lysates from the first plate or strip well to a new well for binding any remaining gDNA from the lysates.
3. Incubate for 30 minutes at room temperature to allow binding of gDNA.

   Note: You may shorten the incubation time to 1 minute, but a shorter incubation time may lead to a reduced yield of gDNA.
4. Repeat Steps 2 and 3 for a total of up to 5 ChargeSwitch® gDNA plate or strip wells.

   Note: The amount of gDNA bound to replica wells continuously decreases each time the lysate is reused for another well.
5. Proceed to **Washing Step**, below.

Washing Step

During the washing step, mix by pipetting up and down twice after addition of wash buffers to improve removal of contaminants.

1. Aspirate the liquid from wells without scraping the well sides.
2. Add 120 µl Wash Buffer (W10) to the wells. Aspirate Wash Buffer (W10).
3. Add 120 µl Wash Buffer (W12) to the wells. Aspirate Wash Buffer (W12) completely.
4. Proceed to **Performing PCR** (next page) or any downstream application.

Note: You may seal and store the gDNA plate or strip with bound gDNA at room temperature, 4ºC, or -20ºC until further use. You may use the gDNA bound to the plate or strip directly for your applications without quantitation.

Optional: Elution Step

We recommend performing downstream applications such as PCR reactions directly in the ChargeSwitch® plate or strip without eluting the purified gDNA. However, if your downstream applications cannot be performed in a 96-well plate format or the 0.2 ml strip-well tube, elute the gDNA as described below.

1. Add 100 µl Elution Buffer (E5) to each ChargeSwitch® well. Mix by pipetting up and down 5 times.
2. Cover the plate wells with PureLink™ Foil Tape (cat. no. 12261-012) or cap the strip-well tube and incubate at 55°C for 2 hours. Mix again by pipetting up and down 5 times.

   Note: You can store the gDNA in the same ChargeSwitch® gDNA plate or strip. Transfer of eluted gDNA is not necessary.
3. Store the eluted gDNA at 4 °C (short-term storage) or -20°C (long-term storage) until further use.

Note: If you wish to quantitate the eluted, purified gDNA, use Quant-iT™ DNA Assay Kit (cat. no. Q33120). Using UV measurements (A260/A280 nm) is **not recommended** as the UV method is inaccurate for low DNA concentrations.

Performing PCR

The gDNA isolated using the ChargeSwitch® gDNA Kits are suitable for use in PCR and qPCR reactions without the need to perform any additional steps. Invitrogen offers a wide variety of PCR and qPCR products (visit www.invitrogen.com for details).

Using gDNA bound to the plate

Follow the guidelines for the PCR reagents you are using. When preparing the PCR mastermix, take into account that the DNA bound to the ChargeSwitch® gDNA well does not add volume to the PCR reaction. Add your prepared PCR mastermix to the wells after removal of the last Wash Buffer (W12). Seal your plate wells with the provided Plate Sealer and perform PCR by incubating the plate in a thermocycler suitable for 96-well plates and equipped with heated lids. For the 8-well strips, we have provided the flat caps suitable for real-time PCR direct in the 0.2 ml tube. The gDNA polypropylene plates and strips are compatible with PCR cyclers from all major suppliers.

Using eluted gDNA

Use up to 10 µl of the purified, eluted gDNA sample to perform PCR in a 25-50 µl reaction volume. Follow the guidelines of the PCR reagents you are using. For qPCR reactions, use optical tubes or plates that are compatible for the PCR cycler of your choice.

Troubleshooting

Problem	Cause	Solution
Low DNA yield	Incomplete binding	Perform gDNA binding for additional 30 minutes.
DNA degraded	DNase contamination	Follow the guidelines on page 2 to prevent DNase contamination.
High level of background fluorescence when performing qPCR using SYBR® Green	Too much gDNA used as PCR template	Use less gDNA as starting material for qPCR. Do not use more than 2 x 10^4 cells or more than 10 µl blood or cell-free samples as starting material.
Poor resolution in STR profiles or the appearance of 'shouldered peaks'	Too much gDNA used for STR analysis	Use less gDNA as starting material for STR profiles. Do not use more than 2 x 10^4 cells or more than 10 µl blood of cell-free samples as starting material.

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