User Guide

SiDirect Cell-free DNA Isolation Kit

For isolation and purification of cell-free DNA
from plasma and serum samples.

Version **210101**
Catalog **K0304-SMP**
K0304-050

7964 Arjons Dr., Ste G
San Diego, CA 92128
Tel.: (858) 689-8808
Website: www.oceannanotech.com
Email: info@oceannanotech.com

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Product description

The *SiDirect* Cell-free DNA Isolation Kit is designed to isolate circulating, cell-free DNA (cfDNA) from plasma and serum. Our magnetic bead technology and optimized chemistries enable efficient, consistent, and scalable isolation of cfDNA from plasma or serum. Using this kit, cfDNA is isolated in high yields, and are suitable for direct downstream applications such as PCR, Real-time PCR, and next-generation sequencing.

Contents and storage

Reagents have a one (1) year expiration date from date of purchase when stored properly. This kit contains beads and solutions sufficient for the specified number of isolations based on the specified input plasma or sample volume.

SiDirect Cell-free DNA Isolation Kit Catalog no.	5 preps [3] K1903-SMP	50 preps [3] K1903-050	Storage
SiDirect Beads [1]	1 ml	1 ml	2-8°C
Lysis/Binding Buffer [1]	10 ml	70 ml	15-30°C
Wash Buffer 1 [1]	10 ml	100 ml	15-30°C
Wash Buffer 2 [1,2] *Customer adds ETOH*	2 ml	20 ml	15-30°C
Elution Buffer [1]	1 ml	5 ml	15-30°C

Note. Some reagents are provided more than the amount required.

[1] *SiDirect* Beads are also available as Catalog no. A1902 and Lysis/Binding Buffer is available as Catalog no. R1226 and Wash Buffer 1 is available as Catalog no. R2335 and Wash Buffer 2 is available as R2300, and Elution Buffer is available as R0510.

[2] Prior to first time use, add the required amount of alcohol as indicated on the label of each bottle, then check the box and mix well.

[3] The number of preps or isolations are based on a 1 ml plasma or serum sample.

Required materials not supplied

Item	Source	Catalog no.
Ethanol, absolute (100%)	Fisher Scientific	BP291914
Proteinase K, lyophilized	Gold Biotech	P-480-100
Micro- and conical tubes (1-15 ml)	Various	Not applicable
Magnetic separator for micro-tubes	Ocean NanoTech	MMS
Magnetic separator for conical tubes	Thermo Fisher	DynaMag-15
Vortex mixer	Various	Not applicable
Heat block for tubes	Various	Not applicable
Rotator (End-Over-End Mixer)	Thermo Fisher	HulaMixer
Pipettors and tips	Various	Not applicable

Principle

The magnetic bead technology and solutions in this kit are used in a simple separation protocol to isolate cfDNA from human serum or plasma: proteinase K solution is first added to the sample, followed by incubation with Lysis/Binding solution and beads. The beads bind to cfDNA in the sample and are easily separated using a magnet. Magnetic separation facilitates efficient washing and elution of the isolated cfDNA.

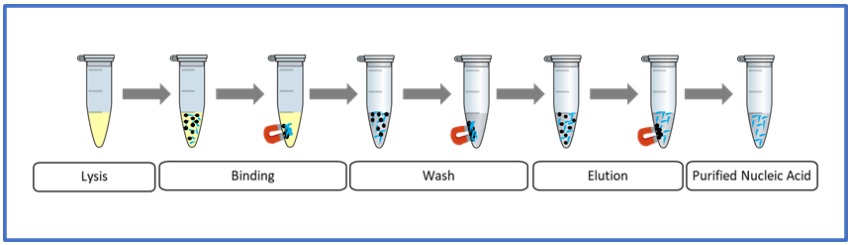

Figure. Illustration showing magnetic separation of cfDNA from plasma sample using the SiDirect Cell-free DNA Isolation Kit.

Procedural guidelines

- Read the USER GUIDE and make sure all the directions are followed and all recommended volumes are used as indicated.
- Prior to use, bring all kit components to room temperature.
- All vortex steps should be performed at maximum speed to ensure mixing.
- *SiDirect* Beads should be resuspended to a homogenous suspension prior to use.

Before you begin

Prepare working buffers

Prior to first time use, add ethanol (100%) as indicated on the label of each bottle(s) of Wash Buffer 2 to obtain a working solution. Check the box on the label and mix well by inverting 10 times.

Prepare proteinase K solution

If plasma was collected into a Streck Cell-Free DNA BCT® or LBgard® Blood Tube, prepare enough proteinase K solution at 20 mg/ml (not provided) by dissolving lyophilized proteinase K powder in 50 mM Tris (pH 8.0). Then divide the stock solution into small aliquots and store at -20°C. Alternatively, a 20 mg/ml proteinase K solution can be purchased from Gold Bio (Catalog no. P-480-SL2) or a different supplier.

Prepare plasma sample

1. Centrifuge blood sample at 2000 x g for 10 minutes at 4°C.
2. Transfer the plasma to a sterile tube and centrifuge plasma sample at 16,000 x g for 10 minutes at 4˚C or 6,000 x g for 30 minutes at 4˚C.
3. Transfer the plasma to a new sterile tube. The plasma is ready to be used. Alternatively, if you do not intent to carry out cfDNA isolations on the same day you prepare the plasma, store the prepared plasma sample at 4°C for up to 24 hours or -20°C for long-term storage.

Protocol: Isolate cfDNA manually from large volumes of plasma or serum samples

The following protocol is for cfDNA isolation from 1 ml plasma or serum. To process plasma volume larger than 1 ml, proportionally increase volumes of all reagents, except those of the Wash Buffers. Protocol has been validated with plasma or serum collected in K_2.EDTA BCT, Streck Cell-Free DNA BCT, and LBgard Blood Tubes. For higher cfDNA yields from tubes other than EDTA tubes prepare and treat plasma or serum samples with Proteinase K (20 mg/ml).

(Optional) Treat plasma samples with proteinase k

Proteinase K treatment is required if you collect blood samples in Streck Cell-Free DNA BCT or LBgard Blood Tubes. Otherwise, proceed directly to the Lyse Sample and Bind *SiDirect* Beads to DNA.

A. Pipet 1 ml plasma or serum sample to a microcentrifuge tube.
B. Add 15 ul Proteinase K (20 mg/ml) (not provided in kit). Then mix well and incubate at 60°C for 20 minutes.
C. At the end of the 20-minute incubation, cool the sample to room temperature by placing them on ice for 5 minutes.

Lyse sample and bind *SiDirect* beads to cfDNA

1. Pipet 1 ml plasma sample to a 15-ml conical tube.
2. Add 1.25 ml Lysis/Binding Buffer and 15 ul *SiDirect* Beads. Briefly vortex to mix, then place the mixture in a rotator (end-over-end) for 10 minutes to bind cfDNA to beads.
3. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

Wash *SiDirect* beads with wash buffers

4. Remove the tube from magnetic separator. Add 1 mL of Wash Buffer 1 and vortex for 30 seconds.

   If you have a magnetic separator capable of holding 1-2 ml microtubes, transfer the resuspended beads to a microtube at this step to facilitate small volume elution later.

5. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
6. Repeat Steps 4-5 for a second wash with the Wash Buffer 1.
7. Remove the tube from magnetic separator, add 1 mL of Wash Buffer 2 and vortex for 30 seconds.
8. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
9. Repeat Steps 7-8 for a second wash with Wash Buffer 2.
10. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 5 minutes. Remove any visible supernatant without disturbing the beads.

    Do not over dry beads as it may result in loss of cfDNA.

Elute the cfDNA

11. Remove the tube from magnetic separator, add 10-50 ul of Elution Buffer and vortex for 5 minutes.
12. Briefly centrifuge tube to bring sample to bottom of the tube. Then place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.
13. Carefully transfer the supernatant containing the purified cfDNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

    The purified cfDNA is ready for immediate use. Alternatively, you can store the DNA at 4˚C for up to 24 hours or -20˚C for long-term storage.

Protocol: Isolate cfDNA manually from small volumes of plasma and serum samples

The following protocol is for cfDNA isolation from 100 ul of plasma or serum sample. To process samples greater than 100 ul, proportionally increase the volumes of only the Lysis/Binding Buffer. This protocol has been validated for plasma and serum volumes up to 600 ul collected in K_2.EDTA BCT, Streck Cell-Free DNA BCT, and LBgard Blood Tubes. For higher cfDNA yields from tubes other than EDTA tubes prepare and treat plasma or serum samples with Proteinase K (20 mg/ml).

(Optional) Treat plasma samples with proteinase k

Proteinase K treatment is required if you collect blood samples in Streck Cell-Free DNA BCT or LBgard Blood Tubes. Otherwise, proceed directly to the Lyse Sample and Bind *SiDirect* Beads to DNA.

A. Pipet 100 ul plasma or serum sample to a micro tube.
B. Add 10 ul Proteinase K (20 mg/ml) (not provided in kit). Then mix well and incubate at 60°C for 20 minutes.
C. At the end of the 20-minute incubation, cool the sample to room temperature by placing them on ice for 5 minutes.

Lyse sample and bind *SiDirect* beads to cfDNA

1. Pipet prepared/digested plasma to a sterile micro tube.
2. Add 125 ul Lysis/Binding Buffer and 10 ul *SiDirect* Beads. Briefly vortex to mix, then place the mixture in a rotator (end-over-end) for 10 minutes to bind cfDNA to beads.
3. Briefly centrifuge the tube to bring the mixture to the bottom of the tube. Then place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

Wash *SiDirect* beads with wash buffers

4. Remove the tube from magnetic separator. Add 500 ul of Wash Buffer 1 and vortex for 30 seconds.
5. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.

6. Repeat Steps 4-5 for a second wash with the Wash Buffer 1.
7. Remove the tube from magnetic separator, add 500 ul of Wash Buffer 2 and vortex for 30 seconds.
8. Place the tube on a magnetic separator for 2 minutes or until beads are pelleted against the magnet. Then carefully discard the supernatant without disturbing the beads.
9. Repeat Steps 7-8 for a second wash with Wash Buffer 2.
10. Keep the tube on the magnetic separator with the lid open. Air dry the beads for 3 minutes. Remove any visible supernatant without disturbing the beads.

    CAUTION: *Do not over dry beads as it may result in loss of cfDNA.*

Elute the cfDNA

11. Remove the tube from magnetic separator, add 10-50 ul of Elution Buffer and vortex for 5 minutes.
12. Briefly centrifuge the tube to bring the sample to bottom of the tube. Then place the tube on the magnetic separator for 2 minutes or until the solution clears and beads are pelleted.
13. Carefully transfer the supernatant containing the purified cfDNA without disturbing the pellet to a clean, labeled microcentrifuge tube.

    NOTE: *The purified cfDNA is ready for immediate use. Alternatively, you can store the DNA at 4˚C for up to 24 hours or -20˚C for long-term storage.*

Troubleshooting

Observation	Possible cause	Recommendation actions
Lower yield than expected	The sample contains low levels of cfDNA.	Increase the starting sample volume.
Variations in cfDNA yield from donor to donor	Levels of cfDNA can range from 1 to 100 ng/mL of plasma or serum depending on the donor.	For samples containing low levels of cfDNA, increase the starting sample volume.

Yield and quality measurements

cfDNA yield

We recommend using the Agilent™ High Sensitivity DNA Analysis Kit (Catalog no. 5067-4626) to quantify the cfDNA fraction. cfDNA is fragmented dsDNA with a major peak around 170 bp.

We recommend using the Invitrogen™ Qubit™ dsDNA HS Assay Kit (Catalog no. Q32855) for total cfDNA yield and quantification. This assay is specifically designed for use with the Qubit Fluorometer but can be used with any fluorometer or fluorescence plate reader.

Document History

Version	Person	Description of Change
210101	Luis M	Issued

Warranties and Disclaimers

The Ocean NanoTech products ("Product") is warranted to operate or perform in conformance with published Product specifications at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by professionally trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper, and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

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