XCF™ COMPLETE Exosome and cfDNA Isolation Kit (for Serum & Plasma)

Cat# XCF100A-1

User Manual

Store kit components at +4°C and +25°C

Version 1
2/2/2017

Contents

Product Description

Building on SBI's popular family of ExoQuick® products for exosome isolation, the XCF™ COMPLETE Exosome and cfDNA Isolation Kit (Cat # XCF100A-1) is a simple, but powerful way to isolate exosomal DNA AND cell-free DNA (cfDNA) from a single serum or plasma sample with <30 minutes of hands-on time. The combined isolation of exosomal and cfDNA will provide researchers with additional analytic capabilities to comprehensively understand the landscape of DNA biomarkers present in their samples, and will help speed identification of novel biomarkers in many different areas of translational research.

The XCF™ COMPLETE Exosome and cfDNA Isolation Kit is a complete kit, containing enough reagents for isolation of both exosomal DNA and cfDNA from 20 different serum or plasma samples, with a volume requirement as low as 500 µL per sample. The kit also offers flexibility with respect to sample processing, there are sufficient reagents to allow processing of a single type of sample DNA: up to 40 exosomal DNA or 40 cfDNA samples with a single kit.

List of Components

Item	Volume	Storage Temperature
Reagent A	0.5 mL	4°C
ExoQuick	6 mL	4°C
DNA Binding Buffer	40 mL	25°C
Concentrated Wash Buffer	15 mL	25°C
Elution Buffer	3 mL	25°C
Spin Columns	40	25°C
Collection Tubes	40	25°C

Storage and Safety Information

Please see the above table for the storage temperatures for each kit component. The expiration date for this kit is 1 year after receipt of the product, if the components are stored properly.

DNA Binding Buffer contains a strong protein denaturant and should be handled with care. The protein denaturant forms reactive compounds when combined with bleach. Therefore, care must be taken to properly dispose liquids containing DNA binding buffer. If there is a spill of liquid containing DNA binding buffer, clean the affected area with suitable laboratory detergent and water. Do not use bleach (sodium hypochlorite) to clean the spilled area.

Human serum or plasma is considered potentially infectious. All necessary precautions should be taken when working with the blood samples. If there is a spill of liquid containing potentially infectious agents, clean the affected area with suitable laboratory detergent and water first, rinse well to remove any trace of the DNA Binding Buffer (if applicable to the fluid spilled) and then clean the area with 1% (v/v) sodium hypochlorite (bleach).

Protocol

Note: For the centrifugation steps described below, please calculate "rpm" for a given "g" based on your centrifuge model and rotor before starting the experiments.

A) Isolation of exosomes from plasma or serum

1. Thaw 500 µL of plasma or serum on ice. If the sample volume is less than 500 µL bring the volume of the sample up to 500 µL using 1xPBS after the sample thaws.

   Note: Cellular genomic DNA may be released from lysing and/or lysed white blood cells following blood draw. When blood samples are processed, precautions should be taken to stabilize white blood cells minimizing cell-free circulating DNA contamination by cellular genomic DNA in the samples.

2. Centrifuge at 3,000 x g for 15 minutes to remove cells, apoptotic bodies, and cell debris and collect supernatant (**Optional:** you may pass the supernatant through 0.22 µm filter to remove micro-particles that are larger than 220 nm from your sample).

3. Vortex Reagent A solution, and add 12 µL of Reagent A to the supernatant from Step 2, mix well by vortexing for 10 seconds, and then incubate at 55^{o}C for 10 minutes.

   Note: Treatment with Reagent A will reduce not only the amount of non-exosomal protein contaminants, but also the amount and/or size of some exosome surface markers.

4. Let the mixture cool down to room temperature. Combine the mixture from Step 3 with 128 µL of ExoQuick.

5. Mix well by vigorous inversion several times.

6. Place at 4^{o}C for 30 minutes.

7. Centrifuge at 13,000 g for 10 minutes.

8. Aspirate the supernatant to separate it from the pellet. Save the supernatant (see Section B below) and continue to the next step with the exosomal pellet.

9. Resuspend the exosome pellet in 500 µL of 1xPBS.

10. The dissolved exosomes can then be used to extract exosomal DNA using the steps below (Section C).

B) Isolation of cell-free circulating DNA (cfDNA) (continued from Step 8, above)

11. Add 35 mL of pure ethanol (99-100%) to 15 mL of the Concentrated Wash Buffer to prepare 50 mL of Working Wash Buffer that contains approximately 70% ethanol and 30% wash buffer (the Working Wash Buffer should be kept tightly sealed and can be stored at 15-25^{0}C). This buffer will be used in Section C, below as well.

12. Add 1,000 µL of DNA Binding Buffer to approximately 630 µL of supernatant from Part A, Step 8, and mix well by vortexing for 10 seconds.

13. Transfer 600 µL of the mixture from Step 12 into a spin column assembled with a collection tube. Centrifuge for 2 minutes at 3,300 x g. Discard the flow-through and reassemble the spin column with the same collection tube.

14. Repeat Step 13 to transfer 600 µL of the remaining mixture from Step 12 into the spin column.

15. Repeat Step 14 to transfer any remaining mixture from Step 12 into the spin column.

16. Add 600 µL of the Working Wash Buffer to the spin column assembled with a collection tube and centrifuge for 1 minute at 3,300 x g. Discard the flow-through and reassemble the spin column with the same collection tube.

17. Repeat Step 17 one more time (i.e., for a total of two washes).

18. Dry Spin before cfDNA elution (important): To remove any residual Working Wash Buffer, centrifuge the spin column from the Step 17 for 2 minutes at 13,000 x g. Discard the collection tube.

19. Transfer the spin column from Step 18 to a 1.5mL Eppendorf tube (not included). Add 50 µL of Elution Buffer to the spin column and let stand at room temperature for 2 minutes. Centrifuge the spin column together with the Eppendorf tube for 1 minute at 400 x g followed by 2 minutes at 5,800 x g.

20. To increase recovery yield, transfer the eluted buffer back to the spin column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x g, followed by 2 minutes at 5,800 x g.

21. Measure the rough, approximate concentration and amount of the isolated cfDNA using NanoDrop and use more accurate measurements for a better estimate of cfDNA concentration. Due to the low concentration and small fragment size of the isolated cfDNA, both the OD reading and the ratio of A260 to A280 may not be accurate. For an accurate estimate of cfDNA, we recommend using high sensitivity DNA quantitation protocols with a Qubit or Agilent BioAnalyzer. The yield of cfDNA will vary from sample to sample depending on the source of the blood sample, expect amounts in the nanogram range.

C) Isolation of DNA from exosomes

1. Prepare exosome stock solution: Exosomes are obtained from Part A of the protocol above. Exosomes should be stored in 1xPBS, and a volume of ~500 µL is required for further steps, below. If the volume of the exosome solution of interest is less than 500 µL, bring the volume of the exosome solution up to 500 µL using 1xPBS.

2. Add 1000 µL of DNA Binding Buffer to 500 µL of the exosome solution from Step 1. Mix well by vortexing for 10 seconds.

3. Transfer approximately 600 µL of the mixture from Step 2 into a spin column assembled with a collection tube. Centrifuge for 2 minutes at 3,300 x g. Discard the flow-through and reassemble the spin column with the same collection tube.

4. Repeat Step 3 to transfer 600 µL of the remaining mixture from Step 2 into the spin column.

5. Repeat Step 4 to transfer any remaining mixture from Step 2 into the spin column.

6. Add 600 µL of the Working Wash Buffer to the spin column assembled with a collection tube and centrifuge for 1 minute at 3,300 x g. Discard the flow-through and reassemble the spin column with the same collection tube.

7. Repeat Step 7 one more time (i.e., for a total of two washes).

8. Dry Spin before exosomal DNA elution (important): To remove any residual Working Wash Buffer, centrifuge the spin column from Step 7 for 2 minutes at 13,000 x g. Discard the collection tube.

9. Transfer the spin column from Step 9 to a 1.5mL Eppendorf tube (not included). Add 50 µL of Elution Buffer to the spin column and let stand at room temperature for 2 minutes. Centrifuge the spin column together with the Eppendorf tube for 1 minute at 400 x g followed by 2 minutes at 5,800 x g.

10. To increase recovery yield, transfer the eluted buffer back to the spin column and let stand at room temperature for 2 minutes. Centrifuge for 1 minute at 400 x g, followed by 2 minutes at 5,800 x g.

11. Measure the rough, approximate concentration and amount of the isolated cfDNA using NanoDrop and use more accurate measurements for a better estimate of cfDNA concentration. Due to the low concentration and small fragment size of the isolated cfDNA, both the OD reading and the ratio of A260 to A280 may not be accurate. For an accurate estimate of cfDNA, we recommend using high sensitivity DNA quantitation protocols with a Qbit or Agilent BioAnalyzer. The yield of cfDNA will vary from sample to sample depending on the source of the blood sample, expect amounts in the nanogram range.

Next Steps and Related Products

Application	Related Products	Website links
Protein Characterization of Exosomes		
Western blotting	Exosome antibodies	https://www.systembio.com/microrna-research/exosome-antibody/exoab
Antibody Arrays	ExoCheck™ Assays	https://www.systembio.com/microrna-research/exosome-antibody-arrays
Quantification of Exosomes		
Quantification of exosomes	FluoroCet assay	https://www.systembio.com/quantitate-exosomes/fluorocet
Quantification of exosomes	ExoELISA-ULTRA assay	https://www.systembio.com/quantitate-exosomes/exoelisa-ultra
RNA extraction from Exosomes		
RNA extraction and profiling	SeraMir™ kits	https://www.systembio.com/microrna-research/seramir-exosome-rna-profiling/overview

Example Data and Applications

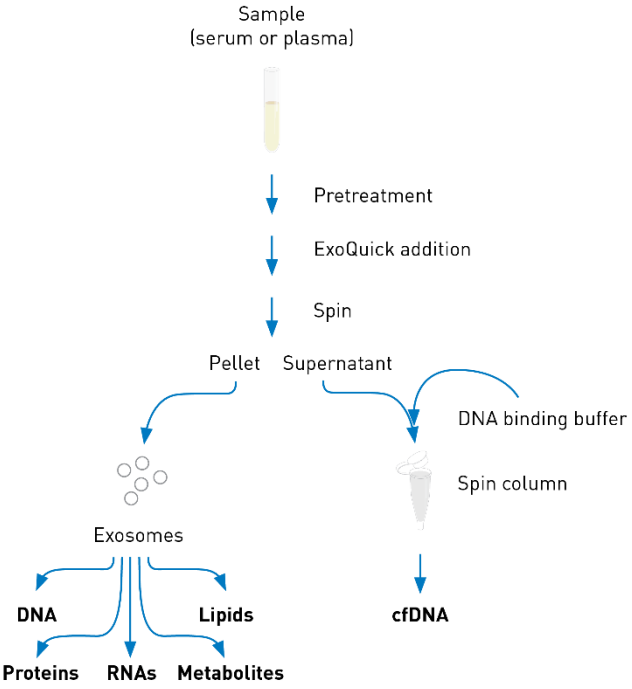

Figure 1. Workflow summary of XCF™ COMPLETE Exosome & cfDNA Isolation Kit for complete DNA isolation from serum and plasma biofluids

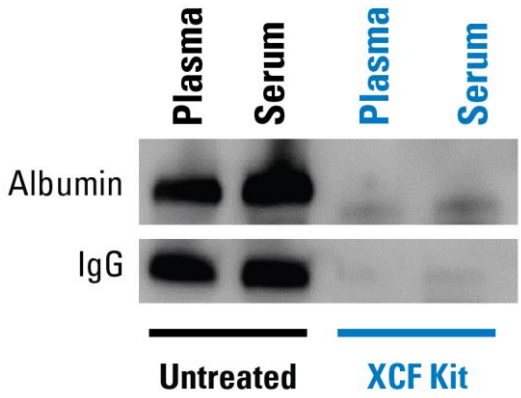

Figure 2. Western blots of co-purifying human albumin (~67kD) and heavy-chain IgG (~50kD) comparing untreated serum and plasma (left two lanes) versus the same samples processed using the XCF Kit (right two lanes), show much less protein carryover in the samples processed using the XCF Kit. For each lane, 20 µg of total protein were loaded.

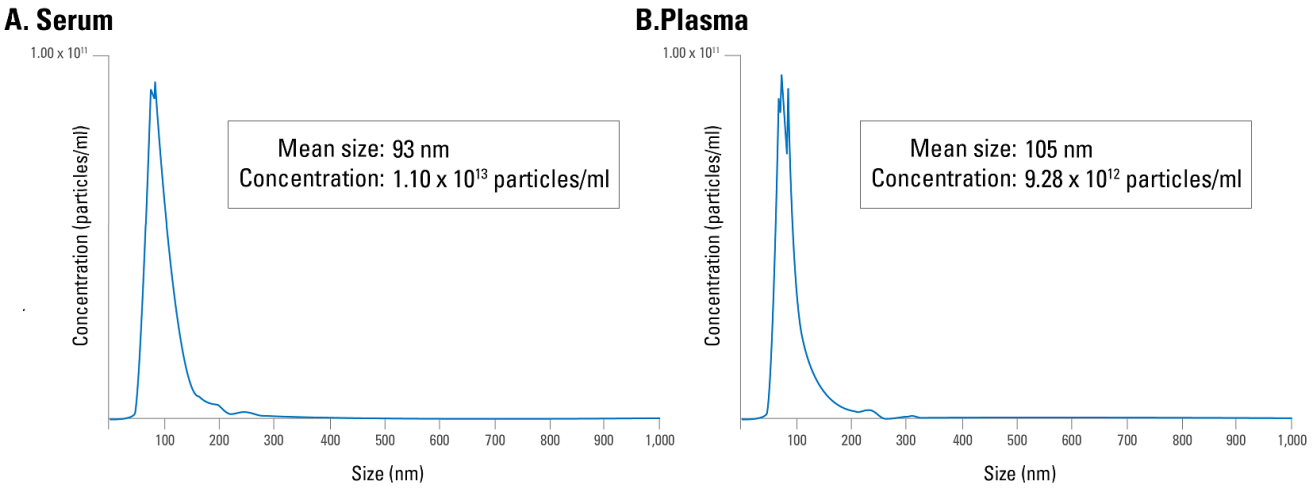

Figure 3. NanoSight NTA data for extracellular vesicles isolated from 500 µL of serum and plasma samples prior to DNA extraction using XCF COMPLETE Exosome and cfDNA Isolation Kit, showing representative size distribution and high concentration of particles.

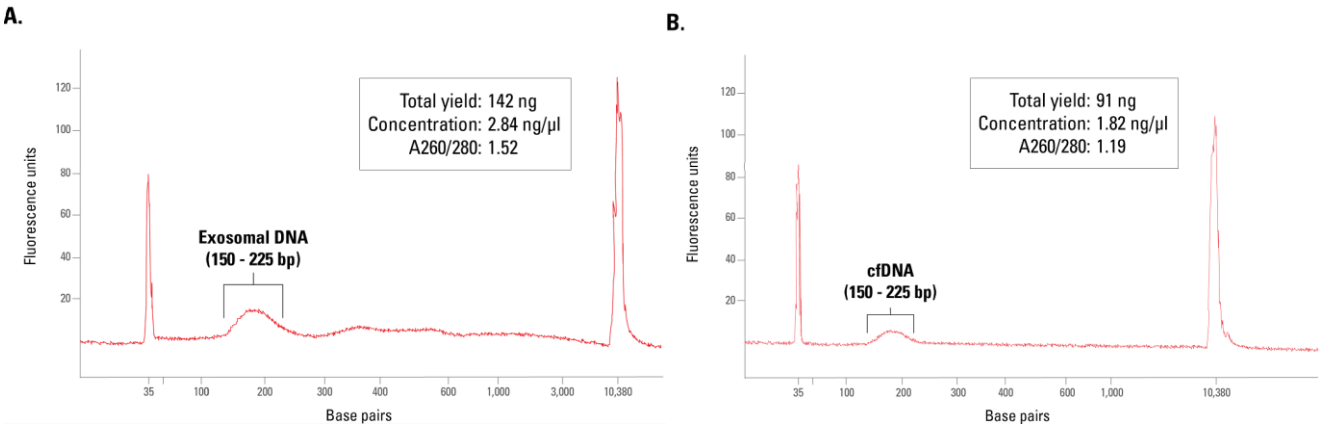

Figure 4. Agilent Bioanalyzer data showing exosomal DNA (Fig. 4A) and cfDNA (Fig. 4B) profiles from 500 µL of human serum sample. A notable peak around ~166bp is seen in both cfDNA and exosomal DNA, which is consistent with cfDNA sizes reported in literature[1]. Concentration, yield, and quality of exosomal DNA and cfDNA are reported.

References

Lo YM, et al. (2010) Maternal plasma DNA sequencing reveals the genome-wide genetic and mutational profile of the fetus. *Sci Transl Med* 2(61):61ra91 2010

Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site: **http://www.systembio.com**

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Licensing and Warranty Statement

Limited Use License

Use of the XCF™ COMPLETE Exosome & cfDNA Isolation Kit (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

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- This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

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SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This

limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.

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