XCF™ Exosomal DNA Isolation Kit

Cat# XCF200A-1

User Manual

Store kit components at +25°C

Version 1
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Contents

Product Description

The XCF™ Exosome DNA Isolation Kit (Cat # XCF200A-1) is a simple, and efficient way to isolate exosomal DNA from numerous exosome preparations (e.g. ExoQuick, ultracentrifugation, OptiPrep, immunoaffinity capture, etc.) with <10 minutes of hands-on time.

The kit contains the components required to isolate exosomal DNA from 20 different exosome preps.

List of Components

Item	Volume	Storage Temperature
DNA Binding Buffer	20 mL	25°C
Concentrated Wash Buffer	7.5 mL	25°C
Elution Buffer	1.5 mL	25°C
Spin Columns	20	25°C
Collection Tubes	20	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.

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.

Isolation of DNA from exosomes

1. Prepare exosome stock solution using 1xPBS to a volume of 500 µL. Exosomes are provided by the user, the exosomes should be at a concentration of ~5-11 x 10^{12}/mL.
2. Add 1000 µL of DNA Binding Buffer to 500 µL of the exosome solution from Step 1. 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 PBS. 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 with any remaining mixture from Step 2.
6. Add 17.5 mL of pure ethanol (99-100%) to 7.5 mL of the Concentrated Wash Buffer to prepare 25 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°C).
7. 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.
8. Repeat Step 7 one more time (i.e., for a total of two washes).
9. Dry Spin before the DNA Elution (important): To remove any residual Working Wash Buffer, centrifuge the spin column from the Step 8 for 2 minutes at 13,000 x g. Discard the Collection tube.
10. 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.
11. 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.
12. 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

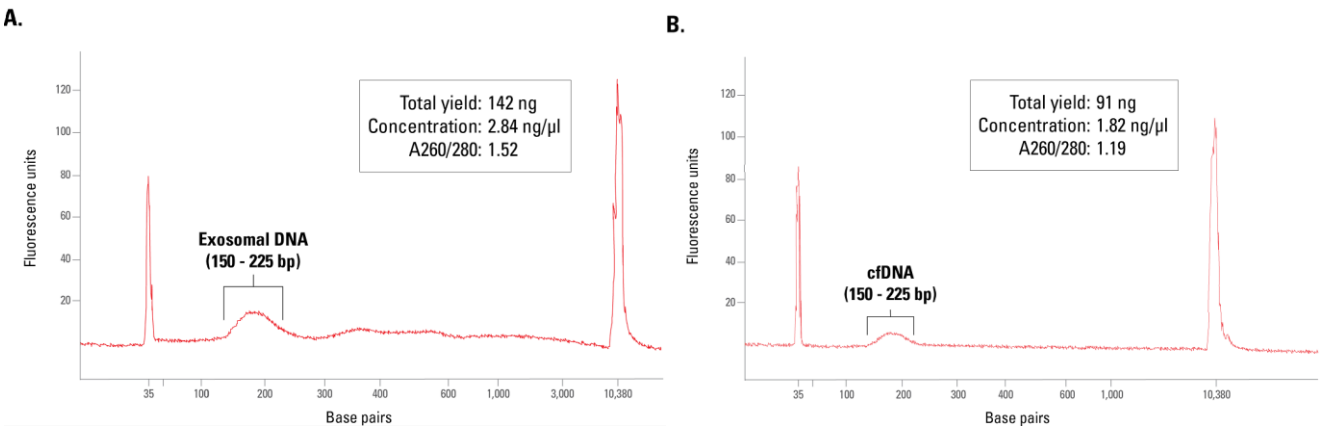

Figure 1. Agilent Bioanalyzer data showing exosomal DNA (Fig. 1A) and cfDNA (Fig. 1B) profiles from 500 µL~~ul~~ of human serum ~~sample~~. A notable peak around ~166bp is seen in both cfDNA and exosomal DNA, which is consistent with cfDNA sizes reported in literature[1]. Concentration, yield, and quality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