cfMAX™ cfDNA Isolation System

Cat # CFMAX100A-1

User Manual

Storage: Magnetic Beads, 2-8°C (Do NOT freeze!);
Buffers, room temperature

Version 2
11/5/2019

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Product Description

Reliable insights from cfDNA start with high-quality, high-yield isolation

While the existence of cell-free DNA (cfDNA) has been known for almost a century[1], it's only in the past decade or so that researchers have been able to recognize and fully exploit the valuable insights to be gained from analyzing cfDNA. In oncology, cfDNA is promising to reduce the need for invasive tissue biopsies by providing insight into the genetic status of solid tumors through a simple blood draw, and in prenatal testing, cfDNA is a powerful screening tool for genetic abnormalities that is reducing the need for invasive amniocentesis procedures.

cfDNA is released into the bloodstream from normal and cancerous apoptotic or necrotic cells and is highly fragmented, with an average length of around 170 bp[2]. By detecting and analyzing cfDNA, as well as extracellular vesicles (including exosomes) and circulating tumor cells, researchers can obtain rich information to aid in the development of tools for the diagnosis, treatment, prognosis and monitoring of cancer.

Isolating cfDNA from plasma and serum can be challenging, as the amount of cfDNA present in individual samples can vary widely[3] and analysis of cfDNA can be complicated by the presence of even low amounts of longer contaminating genomic DNA. With the cfMAX™ cfDNA Isolation System (Cat.# CFMAX100A-1) from SBI, you can overcome these challenges and consistently obtain higher yields of cfDNA than other kits can deliver with a kit optimized for isolation of short DNA fragments. The result is more reliable downstream analysis and greater insights into disease states.

With cfMAX you can:

- Reliably achieve high yields of cfDNA
- Maximize isolation of short DNA fragments while reducing contamination from longer genomic DNA
- Get superior performance compared to competitor kits
- Maximize productivity with the quick and easy magnetic bead isolation workflow

The cfMAX cfDNA Isolation System maximizes your flexibility and accommodates your lab's capabilities:

- Suitable for a range of input volumes
- Compatible with both manual and automated isolation workflows
- Interchangeable with MagMAX™ cfDNA isolation kit in Kingfisher systems

A faster, easier workflow than competitor kits

The cfMAX cfDNA Isolation System uses a fast and simple magnetic bead isolation workflow—lyse your serum or plasma sample with the included cfMAX buffer, add beads, immobilize beads with the magnet, wash the beads, and elute cfDNA.

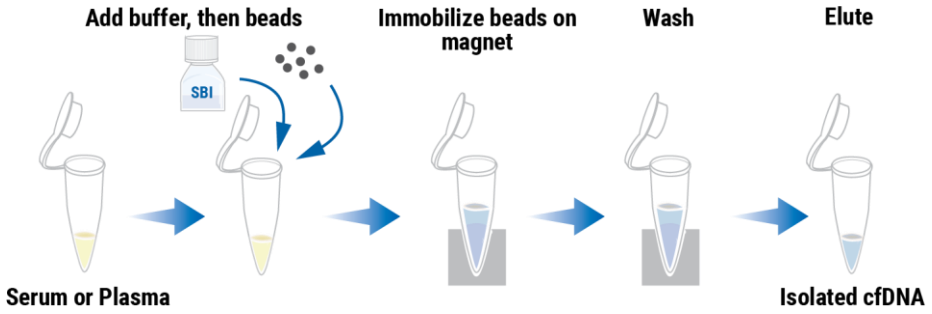

Figure 1. The fast cfMAX workflow.

References

1. Mandel P, Metais P. Les acides nucleiques du plasma sanguin chez l'homme [in French]. *C R Seances Soc Biol Fil*. 1948;**142**:241–243.
2. Johann DJ Jr, *et al*. Liquid biopsy and its role in an advanced clinical trial for lung cancer. *Exp Biol Med (Maywood)*. 2018 Feb;**243**(3):262-271. PMCID: PMC5813874.
3. Babji D, Nayak R, Bhat K, and Kotrashetti V. Cell-free tumor DNA: Emerging reality in oral squamous cell carcinoma. *J Oral Maxillofac Pathol*. 2019 May-Aug; **23**(2): 273–279. PMCID: PMC6714275.

List of Components

Table 1. cfMAX cfDNA Isolation System Components*		
Component	**Qty/Volume**	**Storage Temperature**
Magnetic Beads	2 ml	2-8°C (Do NOT freeze!)
Lysis/Binding Buffer	85 ml	RT
Wash Buffer	40 ml	
Elution Buffer	3 ml	

*Each kit contains enough reagents to process 80 mL of serum or plasma. To purchase bulk volumes, please inquire at services@systembio.com.

Additional Required and Optional Equipment Not Included in Kit

- Pipettes
- Fisher Scientific Vortex Mixer or similar vortexing mixer
- Fisherbrand™ Vortex Mixer accessories: Insert Retainer (cat# 02-215-391)
- Foam inserts set (cat# 02-215-394 or 02-215-386)
- Magnet stand for molecular applications
- 1.5 ml non-stick Eppendorf tube(s)
- Fresh absolute ethanol

Prior to Initial Use

The Lysis/Binding and Wash Buffer are shipped as a concentrate. If precipitate is present in either solution, incubate solution at 37°C for 30 minutes or more. Add absolute ethanol as indicated into the bottle of Lysis/Binding Buffer and Wash Buffer. Mix well and tightly enclose the bottle cap to prevent ethanol evaporation.

- Add 15 ml of fresh absolute ethanol to Lysis/Binding Buffer and mix by inverting gently
- Add 40 ml of fresh absolute ethanol to Wash Buffer and mix by inverting gently
- Prepare fresh 80% ethanol solution prior to each extraction

Starting Material: Both fresh and frozen plasma can be used with the Cell-Free DNA isolation protocol. Fresh plasma, however, tends to have higher yields.

Quantification: We suggest using Bioanalyzer High Sensitivity DNA assay for measuring cfDNA.

Streck Cell-Free DNA BCT Tube(s): Plasma from blood collected with Streck Cell-Free DNA BCT Tube(s) must go through a Proteinase K treatment prior to Cell-Free DNA isolation to ensure optimal yields.

Forgoing Proteinase K treatment may decrease yields by 50%. For EDTA, LBgard tube, Proteinase K treatment can be skipped.

Protocol

Proteinase K Pre-treatment (for plasma sample collected using Streck Cell-free DNA BCT tubes)

Note: If blood was not collected with Streck Cell-Free DNA BCT tube(s) skip this step.

If samples were collected using a Streck Cell-Free DNA BCT tube(s), Proteinase K treatment is required to ensure optimal yields.

Plasma (ml)	Proteinase K	20% SDS Solution
2	30 ul	100 ul
5	75 ul	250 ul
7	105 ul	350 ul
X	0.015X ml	0.050X ml

1. Add the appropriate amount of plasma to an appropriately sized tube(s): For example, use 1.5 ml Eppendorf tubes for 0.5ml to 1ml plasma, 15 ml conical tubes for plasma 2 to 5ml, and 50ml conical tubes for plasma volume larger than 5 ml.

   Note: Using a 50 ml tube(s) for 5 ml or more of plasma is recommended over a 15 ml tube(s). While a 15 ml tube(s) will work it may lead to slightly lower yields.

2. Add 15 µl of Proteinase K (20 mg/ml) for every 1 ml of plasma used.
3. Add 50 µl of 20% SDS solution for every 1 ml of plasma used.
4. Mix by inverting gently 5 times.
5. Incubate at 60°C for 20 minutes.
6. After incubation, place tube(s) on ice for 5 minutes to cool tube(s) to room temperature.
7. Once tube(s) are at room temperature, proceed to Step 1 of the Lysis/Binding section in Protocol.

cfDNA isolation

Before starting the protocol, determine the amount of plasma/serum to be used for extraction and calculate the amounts of buffers and beads needed. Any amount from 500 µl to 8 ml of plasma/serum can be used. Scale buffer and bead volumes accordingly using the table below.

Small (0.2 ml to 7.9 ml) Sample Protocol

Plasma	Lysis/Binding Buffer	Beads solution	Tube size
X (x=ml of plasma)	1.25x	0.025x	Choose by plasma volume
5ml	6.25ml	125ul	15ml or 50ml *
7ml	8.75ml	175ul	50ml

*Using a 50 ml tube(s) for 5 ml or more of plasma is recommended over a 15 ml tube(s). While a 15 ml tube(s) will work it may lead to slightly lower yields.

Lysis/ Binding

8. Add the appropriate amount of plasma/serum to appropriately sized tube(s).
9. Add 1.25 ml of **Lysis/Binding Buffer** for every 1 ml of plasma/serum used. Briefly mix.
10. Add 25 µl of **Magnetic Beads** for every 1 ml of plasma/serum.

    Important: Mix beads well prior to aliquoting. There should be no visible sedimentation at the bottom of the solution after mixing. Beads will settle quickly, so be sure to mix the magnetic bead solution after adding to each sample. Failure to do so may result in inconsistent yields.

11. Vortex or shake tube(s) vigorously for 10 minutes at room temperature to bind the cfDNA to the beads.

    To obtain high yields, ensure that sample/buffer solution is mixed vigorously in the tube(s). A vortexing mixer (speed 7 or higher) with a tube-holder is highly recommended.

12. Place tube(s) into a magnet stand for 2 to 5 minutes, or until solution clears.
13. While keeping the tube(s) on the magnet stand, remove supernatant. Be careful not to remove magnetic particles.
14. Keep tube(s) on magnet stand for 1 more minute, and careful remove any residual liquid.

First Wash

15. Add 1 ml of **Wash Buffer** to the lysis/binding tube(s).
16. Resuspend beads by vortexing for 20 seconds or pipetting up and down 15 times.
17. Transfer magnetic particle suspension into a new 1.5 ml micro tube(s) on magnet stand.
18. Allow beads to attach to magnet stand for 30 seconds.
19. Pipette supernatant from the 1.5 ml tube(s) and use the supernatant to wash the lysis/binding tube(s).
20. Transfer the rest of the magnetic particles in lysis/binding tube(s) to the 1.5 ml tube(s).
21. Keep tube(s) on magnet stand for 30 seconds or until solution is clear.
22. Remove as much buffer as possible using a 1 ml pipette.
23. Tap magnet stand on bench 5 times and remove remaining wash buffer with a 200 µl pipette.
24. Transfer tube(s) to non-magnetic rack and add 1 ml of **Wash Buffer.**
25. Resuspend beads by vortexing for 20 seconds or pipetting up and down 15 times.

26. Centrifuge tube(s) briefly.

    *Centrifuge steps are needed when using vortexing to resuspend beads. Only a brief spin is needed to remove solution from tube(s) lid.

27. Place tube(s) on magnet stand for 30 seconds or until solution is clear.
28. Remove as much buffer as possible using a 1 ml pipette.
29. Tap magnet stand on bench 5 times and remove remaining wash buffer with a 200 µl pipette.

Second Wash

30. Transfer tube(s) to non-magnetic rack and add 1 ml of **80% EtOH.**
31. Resuspend beads by vortexing for 20 seconds or pipetting up and down 15 times.
32. Centrifuge tube(s) briefly.
33. Place on magnet stand for 30 seconds or until solution clears.
34. Remove as much buffer as possible using a 1 ml pipette.
35. Tap magnet stand on bench 5 times and remove remaining EtOH with a 200 µl pipette.
36. Transfer tube(s) to non-magnetic rack and add 1 ml of **80% EtOH.**
37. Resuspend beads by vortexing for 20 seconds or pipetting up and down 15 times.
38. Centrifuge tube(s) briefly.
39. Place on magnet stand for 20 seconds or until solution clears.
40. Remove as much EtOH as possible using a 1 ml pipette and leave cap open.
41. Tap magnet stand with tube(s) on bench 5 times and remove remaining EtOH with a 200 µl pipette.
42. Centrifuge tube(s) briefly and place on magnet stand for 10 seconds.
43. Remove small trace of remaining EtOH with a 20 µl pipette.
44. Leave tube(s) open on magnet stand and allow magnetic particles to air dry for 4-5 minutes.

*Be careful not to over-dry or beads may stick to tube(s).

Elution Step

45. Transfer the microtube(s) to non-magnetic rack and add desired volume of **Elution Buffer** and resuspend beads.

Important: A minimum of 12.5 µl of **Elution Buffer** per ml of plasma is recommended to elute DNA to ensure optimal yields. Typically, 20- 30 µl of Elution Buffer is used per ml of plasma.

46. Vortex or shake tube(s) vigorously for 5 minutes.
47. Centrifuge tube(s) briefly.
48. Place tube(s) on magnetic rack for 10 to 30 seconds.
49. Transfer elute into a new 1.5 ml tube(s).

Example Data and Applications

The cfMAX cfDNA Isolation System extracts more cfDNA than other kits

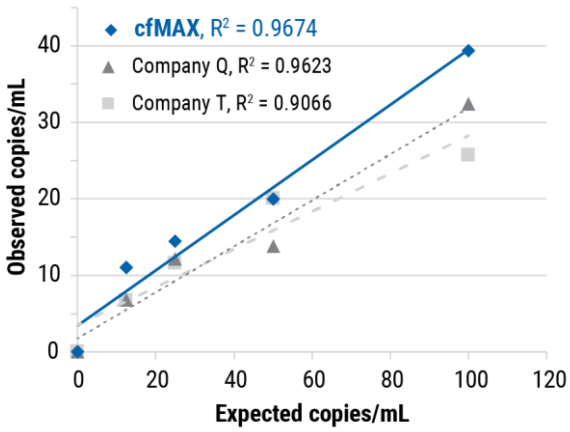

Figure 2. The cfMAX cfDNA Isolation System extracts more cfDNA than other kits. *We added known amounts of a synthetic cfDNA to DNA-depleted plasma replenished with 5 ng of sheared genomic DNA and compared cfDNA isolation efficiency of the cfMAX cfDNA Isolation System to two competitor kits. The cfMAX cfDNA kit more consistently and linearly delivered the expected amount of the spiked-in DNA than the competitor kits (n=3 for each data point).*

cfMAX delivers cfDNA with less contaminating genomic DNA and higher yields of cfDNA than competing kits

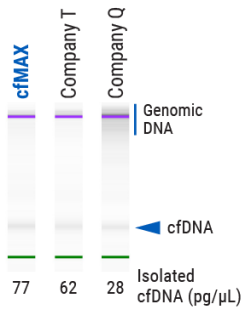

Figure 3. cfMAX delivers cfDNA with less contaminating genomic DNA and higher yields of cfDNA than competing kits. *We isolated cfDNA from 2 mL of plasma using the cfMAX System and two competitor kits. The cfMAX system shows less contaminating genomic DNA than the other kits.*

cfMAX delivers cfDNA with less protein carryover than competing kits

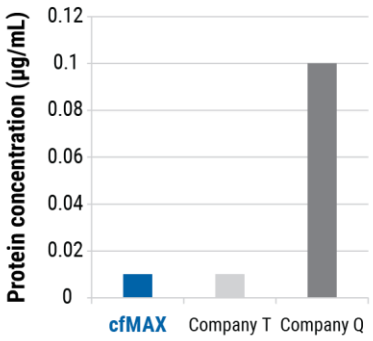

Figure 4. cfMAX delivers cfDNA with less protein carryover than competing kits. *We isolated cfDNA from 2 mL of serum using the cfMAX System and two competitor kits. The cfMAX system shows significantly less protein carryover than Company Q's kit, and similar low amounts of protein carryover as Company T's kit, as determined by Qubit Protein Assay.*

cfDNA isolation with cfMAX is scalable

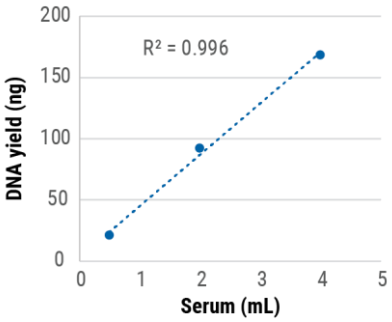

Figure 5. cfDNA isolation with cfMAX is scalable*. We isolated cfDNA from increasing volumes of the same serum sample using cfMAX. Isolation is highly linear across the input sample volumes.*

cfDNA isolation with cfMAX is reproducible

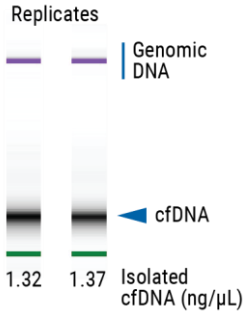

Figure 6. cfDNA isolation with cfMAX is reproducible. *We isolated cfDNA from 2 mL of the same serum sample in duplicate using cfMAX. The similar yields demonstrate the reproducibility of cfMAX.*

cfMAX is optimized for isolating smaller DNA fragments

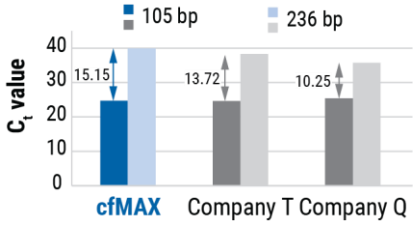

Figure 7. cfMAX is optimized for isolating smaller DNA fragments. *Using 105- and 236 bp fragments of GAPDH, we assessed the efficiency of DNA isolation by cfMAX and the other kits from serum. The DNA isolated by the cfMAX kit shows a much larger difference in Ct values than the DNA isolated from the other kits, indicating that the cfMAX kit more preferentially isolates smaller DNA fragments.*

Technical Support

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