MCLAB
Molecular Cloning Laboratories

User Manual

Version 1.2

Product name: MCMax Cell-Free DNA Extraction Kit

Cat #: MCMAX-100

Size: 1 kit

Description:

MCMax Cell-Free DNA Extraction Kit is designed to purify cell-free DNA (cfDNA) from 0.2–10 ml plasma samples. The kit contains everything necessary for purification, with no preprocessing required. The purified cell-free DNA is of high quality with little genomic DNA contamination, and is ready for use in downstream applications such as qPCR, next generation sequencing and digital PCR.

Components:

Components	Volume (mL)	Storage Temp (°C)
Magnetic beads	1.5	4-8
Lysis buffer	125	RT
Wash buffer	100	RT
Elution buffer	5	RT

Blood Collection:
The cfDNA extraction kit has been optimized for use with samples collected in EDTA tubes and CPD tubes. For Blood collected in Streck Cell-Free DNA BCT, extra proteinase K treatment is needed. Both fresh and frozen plasma can be used with the protocol.

Notes:

- Thaw the frozen plasma sample and centrifuge it at 6000 × g for 30 minutes at 4˚C to remove any residual blood and cell debris.
- Warm up the sample to room temperature before the extraction.
- Prepare fresh 80% EtOH for each time of extraction.

Protocol:

Lysis/ Binding

1. Add 2 mL plasma to 50mL tube. Add 2.5 mL of Lysis/Binding Buffer and 30 µL of well-mixed magnetic bead solution.

2. Vortex or shake tube vigorously for 10 minutes at room temperature. Centrifuge the tube at ~1000 g for 30 second.

3. Place tube into a magnet stand for 5 minutes, or until solution clears. While keeping the tube on the magnet stand, remove supernatant. Keep tube on magnet stand for 1 minute, and remove residual supernatant.

First Wash

4. Add 1000 µL of Wash Buffer to lysis/binding tube. Resuspend beads by pipetting up and down 10 times.

5. Transfer magnetic particle suspension into 1.5 ml tube on magnet stand. Allow beads to attach to magnet stand for 10-30 seconds.

6. Pipette supernatant from 1.5 ml tube and use the supernatant to wash the lysis/binding tube.

7. Transfer the rest of the magnetic particles in lysis/binding tube to the 1.5 ml tube. Keep tube on magnet stand for 2 minutes or until solution is clear. Remove as much buffer as possible using a 1000 µL pipette. Remove remaining wash buffer with 200 µL pipette.

8. Transfer tube to non-magnetic rack and add 1000 µL of Wash Buffer. Resuspend beads by vortexing for 30 seconds. Centrifuge tube briefly. Place tube on magnet stand for 2 minutes. Remove as much buffer as possible using a 1000 µL pipette. Remove remaining wash buffer with 200 µL pipette.

Second Wash

9. Transfer tube to non-magnetic rack and add 1000 µL of 80% EtOH. Resuspend beads by vortexing for 30 seconds. Centrifuge tube briefly. Place on magnet stand for 2 minutes or until solution clears.

10. Remove as much buffer as possible using a 1000 µL pipette. Remove remaining EtOH with 200 µL pipette. Transfer tube to non-magnetic rack and add 1000 µL of 80% EtOH.

11. Resuspend beads by vortexing for 30 seconds. Centrifuge tube briefly. Place on magnet stand for 2 minutes.

12. Remove as much EtOH as possible using a 1000 µL pipette and leave cap open. Remove remaining EtOH with 200 µL pipette. Centrifuge tube briefly and put it back on magnet stand. Use 20 uL pipette to remove as much EtOH as possible. Leave tube open on magnet stand for 2-5 minutes until beads dry.

Sample Elution

13. Transfer microtube to non-magnetic rack and add 15-30 uL of Elution Buffer and resuspend beads. Vortex tube vigorously for 5 minutes.

14. Centrifuge tube briefly and then place it on magnetic rack for 2 minutes. Transfer elute into a new 1.5 ml tube.