AceSeq™ Circulating Cell-Free DNA Extraction Kit

Cat#: AGCF0316

Product Description:

Free-circulating nucleic acids, such as tumor-specific extracellular DNA fragments and mRNAs in the blood or fetal nucleic acids in maternal blood, are present in serum or plasma usually as short fragments, <1000bp(DNA). AceSeq™ Circulating Free DNA Extraction Kit is used for extraction of circulating free DNA (cfDNA) from cell-free biological samples (e.g. plasma). Samples can be rather fresh or frozen (provided that they have not been frozen and thawed more than once). The AceSeq™ Circulating Cell-Free DNA Isolation Kit contains all components which have been optimized for the simple and rapid isolation of small size nucleosomal DNA from plasma/serum. No phenol–chloroform extraction is required. Nucleic acids bind specifically to the AceSeq Mini column, while contaminants pass through. The isolated ccfDNA can be directly used for real time-PCR and DNA library preparation suitable for next generation sequencing.

Application:

Extract high quality cell free DNA from plasma, serum, and urine for Next Generation Sequencing (NGS), Bisulfite Sequencing, PCR, qPCR, ddPCR and other demanding applications.

Kit Contents:

Product	AGCF0316-4	AGCF0316-10	AGCF0316-50
Purification times	4 Preps	10 Preps	50 Preps
Buffer ACL	20 ml	50 ml	250 ml
Buffer ACB*	30 ml	60 ml	300 ml
Buffer DCW1*	4.4 ml	4.4 ml	22 ml
Buffer DCW2*	5 ml	5 ml	10 ml
Proteinase K	50 mg	110 mg	600 mg
Protease Dissolve Buffer	5 ml	10 ml	40 ml
Carrier RNA	100 µg	100 µg	100 µg
Nuclease Free Water	3 ml	10 ml	20 ml
AceSeq CFDNA Mini Columns	4	10	50
2 ml Collection Tubes	8	20	100
Extender Tube	4	10	50
Vac-Connector	4	10	50

Storage and Stability

AceSeq Circulating DNA Kit components are guaranteed for at least one year when stored at room temperature. Proteinase K/Carrier RNA dry powder is preserved at room temperature. After dissolving, Proteinase K/Carrier RNA needs to be stored at -20°C.

T: +1-862-686-2696 | E: info@accegen.com
F: +1-323-978-5598 | Homepage: www.accegen.com

Materials and Equipment to be Supplied by User

* Dilute Buffer ACB with Isopropanol and store at room temperature
* Dilute Buffer DCW1 and Buffer DCW2 with 100% ethanol and store at room temperature
* 15~50ml centrifuge tubes
* Heat block or water bath capable of 60°C
* 100% ethanol
* Add Protease Dissolve Buffer to the Proteinase K, final concentration is 20mg/ml.Store at -20°C.
* Add Nuclease Free Water to the Carrier RNA, Final concentration is 0.2ug/ul. Store at -20°C.

Reagent volume follow the table:

Sample volumes	**1ml**	**2ml**	**3ml**	**4ml**	**5ml**
Proteinase K	100µl	200µl	300µl	400µl	500µl
Buffer ACL	0.8ml	1.6ml	2.4ml	3.2ml	4.0ml
Carrier RNA	5µl	5µl	5µl	5µl	5µl
Buffer ACB	1.8ml	3.6ml	5.4ml	7.2ml	9ml
Buffer DCW1	750ul	750ul	750ul	750ul	750ul
Buffer DCW2	750ul	750ul	750ul	750ul	750ul
100% Ethanol	750ul	750ul	750ul	750ul	750ul

Protocol for 2ml serum or plasma:

This protocol is for purification of circulating DNA and RNA from 2~4ml of serum or plasma.

1. **Pipet • 200µl, or ø 400µl Proteinase K into a 15~50ml centrifuge tube.**

2. **Add • 2ml, or ø 4ml of serum or plasma to the tube, mix thoroughly.**

3. **Add • 1.6ml , or ø 3.2ml Buffer ACL and 5µl of Carrier RNA (1µg) to the tube,**

Close the cap and mix thoroughly by pulse-vortexing for 30s. Incubate at 60°C for 30min.

4. **Add • 3.6ml, or ø 7.2ml of Buffer ACB to the lysate in the tube, Close the cap and mix thoroughly by pulse-vortexing for 30s.** Incubate the lysate-buffer ACB mixture in the tube for 5min on ice.

5. Connect a new AceSeq CFDNA mini column into a new Vac-connector on the vacuum manifold. Insert a new extender tube into the AceSeq CFDNA Mini Column.

6. **Carefully apply the lysate-Buffer ACB mixture from step 4 into the extender tube of the AceSeq CFDNA Mini column.** Switch on the vacuum pump. When all lysates have been drawn through the columns completely, switch off the vacuum pump and release the pressure to 0 mbar. Carefully remove and discard the extender tube.

7. **Apply 750ul Buffer DCW1 to the column,** Leave the lid of the column open,and switch on the vacuum pump. After all of Buffer DCW1 has been drawn through the AceSeq CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar .

8. **Apply 750ul Buffer DCW2 to the column, Leave the lid of the column open,and switch on the vacuum pump.**

After all of Buffer DCW2 has been drawn through the AceSeq CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

9. **Apply 750ul Buffer 100% ethanol to the column, Leave the lid of the column open,and switch on the vacuum pump.** After all of ethanl has been drawn through the AceSeq CFDNA Mini column, switch off the vacuum pump and release the pressure to 0 mbar.

10. Close the lid of the AceSeq CFDNA Mini Column. Remove it from the vacuum manifold, and discard the VacConnector. **Insert the AceSeq CFDNA Mini Column into 2ml collection tube. Centrifuge at full speed (13,000 × g) for 3 minute at room temperature.** Discard the filtrate and reuse collection tube.

11. Place the AceSeq CFDNA Mini column in a new 2ml collection tube. Open the lid, and incubate the assembly at 56°C for 10 min to dry the membrane completely.

12. **Place the AceSeq CFDNA Mini column in a clean 1.5ml collection tube. Carefully apply 30-50µl Nuclease Free Water directly to the center of the column membrane.** Close the lid and incubate at room temperature for 3 minutes.

13. Centrifuge at 13,000 × g for 1 minute at room temperature. Store DNA at -20°C.

Troubleshooting Guide:

1. Low or no recovery

Buffer DCW1/DCW2/ACB did not contain ethanol/Isopropanol: Ethanol/Isopropanol must be added to Buffer DCW1/DCW2/ACB before used. Repeat procedure with correctly prepare Buffer.

Low concentration of target DNA in the Sample: Samples were standing at room temperature for too long. Repeated freezing and thawing should be avoided. Anticoagulants other than EDTA may lead to accelerated DNA degradation.

2. DNA does not perform well (e.g. in ligation reaction)

Salt concentration in eluate too high: Modify the wash step by incubating the column for 5 min at room temperature after adding 650ul of Buffer DCW2, then centriufge or Vacuum.

Eluate contains residual ethanol: Ensure that the wash flow-through is drained from the collection tube and that the column is then centrifuged at >12,000 x g for 1min, then dry.

Inappropriate elution volume used: Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the volume of eluate added to the amplification reaction accordingly.

3. Clogged AceSeq cfDNA Mini Column

Vacuum pressure of 800-900mbar not reached: The vacuum manifold is not tightly closed.

Transfer the remaining sample lysate to a new tube, place the column in a new collection tube and spin it at full speed for 1 min.