Cell-free DNA (cfDNA) Isolation Kit

(100 rxns)

Zellbio GmbH (Germany)

CAT No. ZX-22105-100

www.zellx.de

For use with PCR, real-time PCR, Southern Blotting and RFLP

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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Please read this insert completely prior to using the product.

Background

Cell-free DNAs (cfDNAs) are DNA fragments ranged between 50-1000 bp bound to mononucleosomes, which circulate extracellularly in bloodstream. cfDNAs in plasma originate from hematopoietic cells and contain sequences of entire genomes. Given the broad range of information they provide, cfDNAs are isolated from human blood in clinical and research laboratories to study the state of health and disease of the donors. The degree of fragmentation in cfDNAs depends on several parameters such as the origin of DNA (fetal, tumor, microbial DNA), health of the donor, blood sampling procedure and handling of the sample. They can be potentially used as a non-invasive detection method to evaluate the state and progression of the disease. Apart from their sequences, cfDNAs also carry the information regarding nucleosomal landscape of cell(s)-of-origin and the dynamics of the epigenome.

ZellX® Cell free DNA (cfDNA) isolation Kit is designed for the quick purification of DNA from fresh or frozen (EDTA or citrate) serum or plasma. It works based on the ability of DNA to bind to silica membrane in the presence of high concentrations of chaotropic salts.

The cfDNA molecules bind to the silica-based membrane, and impurities such as proteins and nucleases are removed by thorough washing steps. The DNA fragments are then eluted in Elution buffer and stored at - 20°C.

ZellX® Cell free DNA (cfDNA) isolation Kit uses a special columns designed for high recovery of fragmented DNA in a range of 100-1000 base pairs.

Intended use

The isolated cfDNA has several molecular biology applications. It is ideal for detection of biomarkers in various diseases. It can also be used to analyze fetal DNA from maternal plasma.

Materials supplied in the Kit

Component	*Quantity*
Minispin Columns	100
Reaction tubes	100
Collection tubes	300
Elution Buffer	4 mL
Reaction Buffer	110 mL
Wash Buffer 1	36 mL
Wash Buffer 2	20 mL
Proteinase K (lyophilized)	4 vial

Storage instruction

All reagents should be kept at room temperature (RT) except the vial of lyophilized Proteinase K which must be stored at -20°C upon receipt. If any kit reagent forms a precipitate, warm it up to 55–65 °C until the precipitate dissolves and allow to reach room temperature before use.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips (Nuclease-free)

Vortex and micro-centrifuge (10000 g)

Sterile 1.5 mL micro-centrifuge tubes (Nuclease-free)

Ethanol 96%-100% (we recommend BioUltra for molecular biology, from Sigma-Aldrich Cat. No. 51976)

Isopropanol molecular biology grade

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.

Reagent preparation

i. **Proteinase K working solution:** Resuspend the supplied lyophilized vial of Proteinase K in 1.9 mL of ddH_2O, mix thoroughly until Proteinase K is completely dissolved and store at -20 °C.

ii. **Wash Buffer 1 and Wash Buffer 2:** Add an appropriate amount (according to the buffer label) of molecular biology grade ethanol (96%-100%) [Not provided] to Wash Buffer 1 and Wash Buffer 2 prior to initial use.

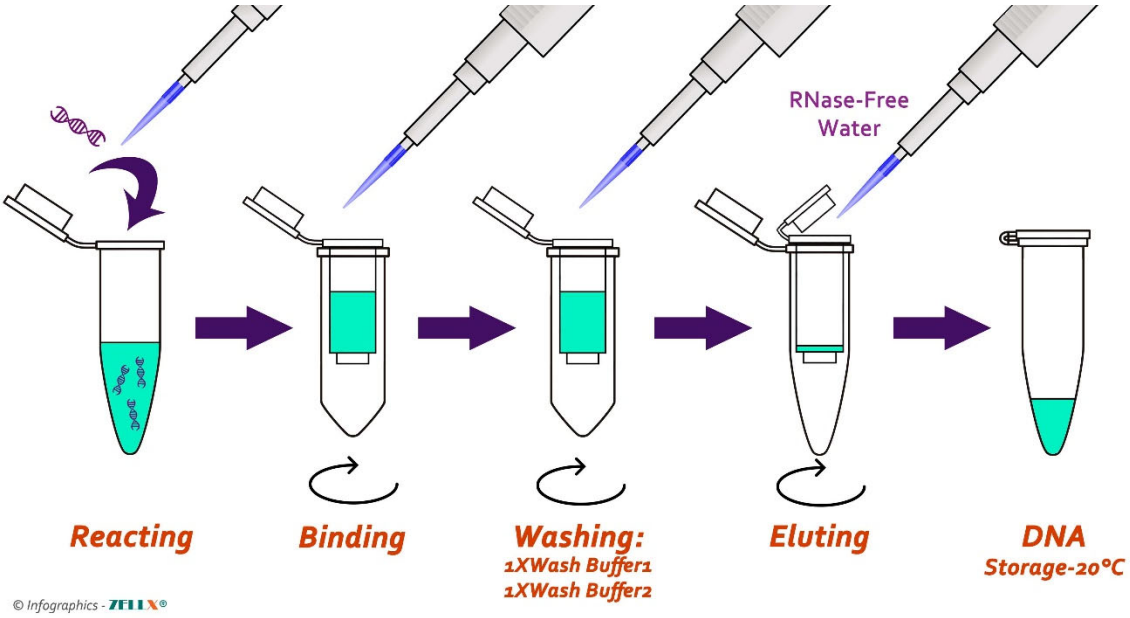
Sample preparation

This kit is designed for the isolation of circulating cell free DNA (ccfDNA) from EDTA or citrate fresh/frozen serum or plasma (heparin Plasma heparin should not be used as heparin can significantly interfere with many downstream applications such as RT-PCR).

Frozen plasma or serum samples should be centrifuged for 2 minutes at 2000 rpm prior to conducting the isolation. Only clear supernatant should be processed, as column clogging may occur if frozen samples are directly processed.

Assay Procedure

1. Place 1000 µL of plasma or serum in a 1.5 mL microcentrifuge tube (not provided) and incubate for 15 minutes at 55 °C. Centrifuge at maximum speed for 5 minutes.
2. Without touching the pellet, transfer the supernatant into a new reaction tube.
3. Add 1000 µL reaction buffer and 75 µL Proteinase K working solution and mix well by vortexing for 10-15 seconds.
4. Incubate at 55 °C for 15 minutes. Invert every 5 minutes.
5. Meanwhile preheat the Elution Buffer to 70 °C.
6. Add 750 µL Isopropanol (not provided) and mix with pipetting.
7. Place a Minispin-column in a 2 mL Collection tube and transfer 700 µL of reaction mixture into the Minispin-column. Centrifuge at 8000 rpm for 30 seconds and discard the flow-through. Repeat this step until all reaction mix is transferred to the Minispin-column, and then centrifuge at full speed at the last transfer step.
8. Place the Minispin-column in a new 2 mL collection tube, add 500 µL of Wash Buffer 1 and centrifuge at 14000 rpm for 60 seconds. Discard the flow-through.
9. Place the Minispin-column in a new 2 mL collection tube, add 500 µL of Wash Buffer 2 and centrifuge at 14000 rpm for 60 seconds. Discard the flow-through.
10. Centrifuge at full speed for an additional 3 minutes to dry the Minispin-column.
11. Place the Minispin-column into a new, labelled 1.5 microcentrifuge tube (not provided) and pipet 30 µL of Elution Buffer (preheated at 70 °C) directly onto the membrane (The tip should not touch the membrane). Close the cap and incubate for 2 minutes at Room Temperature.
12. Centrifuge at maximum speed for 1 minute to elute DNA and store it at -20 °C.
13. To increase the yield, repeat the steps 10 and 11.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