DNAbiotech

Biotechnology is our expertise

DNA Extraction Kit

Liquid-based cytology media and Tissue

(Specifically for HPV)

Catalog no.: DB9822

(50 and 100 prep)

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Diba Noavaran Azma Company

Customer and technical support

If you have any questions, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

About this user manual

It is recommended for first-time users to read the detailed protocol sections of the user manual before using these products. Experienced users, however, may refer to the Protocol-at-a-glance instead. The Protocol-at-glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

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General description

DNAbiotech "**DNAbiotech DB9822 DNA extraction kit"** is optimized for viral DNA extraction (Specifically HPV) from tissue, cultured cells, LBM (LBC), and swabs. It can be used for DNA extraction from serum, plasma, or other body fluids too. Lysis is achieved by incubation of the cells in a solution containing large amounts of chaotropic ions in the presence of proteinase K. Appropriate conditions for binding of DNA to the silica membrane of the corresponding **DNAbiotech Columns** are achieved by the addition of ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Washing steps efficiently remove contaminations with the "**DNAbiotech DB9822 DNA extraction kit"** washing buffers.

Kit specifications

-This kit is designed for the rapid isolation of highly pure genomic DNA from tissue, cultured cells, swabs, LBM (Various types of preservatives), and serum, plasma, urine, or other body fluids.

-The kits allow purification of highly pure genomic DNA with a typical concentration of 30–50 ng per µL.

-The obtained DNA is ready-to-use for subsequent reactions like PCR, Southern blotting, or any kind of enzymatic reaction.

Warranty: The 1st reaction of this product may be used as a free sample and if desired results do not obtain the product could be returned**.**

Quality Control

In accordance with DNAbiotech Co. Management System, each part of the "**DNAbiotechDB9822 DNA extraction kit"** is tested against predetermined specifications to ensure consistent product quality**.**

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Safety Notes

The buffers included in the "**DNAbiotech DB9822 DNA extraction kit"** contain an irritant that is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protectors, and follow standard safety precautions.
Buffer L1 contains chaotropic agents. It can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste**.**

Preparation of samples

Tissue: Excise up to 20 mg of a tissue sample. Either Deparaffinized FFPE (Formalin-Fixed Paraffin-Embedded), frozen or fresh tissue may be used. Place the sample in a nuclease-free microfuge tube.

Deparaffinization

a. Cut sections up to 20 um thick from the interior of an FFPE tissue block using a microtome. Trim off any excess paraffin.
Note: Alternatively, from an FFPE block, cut out up to 25 mg of un-sectioned core. Trim off any excess paraffin. Grind the sample into a fine powder using **liquid nitrogen**.
b. Transfer the sections or ground block into a Nuclease-free micro-centrifuge tube.
c. Add 1 mL of **xylene** to the sample. Mix by vortexing.
d. Incubate at 56°C for 3 minutes.
e. Centrifuge the sample at 14,000 x g (~ 14,000 rpm) for 1 minute.
f. Carefully remove the xylene without dislodging the pellet.
g. Repeat Steps **c** to **f**.
h. Add 1 mL of 96 - 100 % ethanol. Mix by vortexing.
i. Centrifuge the sample at 14,000 x g (~ 14,000 RPM) for 1 minute.
j. Carefully remove the ethanol without dislodging the pellet.
k. Repeat Step **h** to Step **i.**
l. **"Air Dry"** the pellet for about 10 minutes at room temperature.
Note: It is important that all of the ethanol be removed completely.

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Adhesive Cell Cultures: Cells (use ≤ 1 × 10^6 cells) are detached by trypsinization and collected into a 1.5 mL tube. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 mL of PBS once by centrifugation at 2000 rpm for 3 minutes. The supernatant should be discarded and the precipitated cells will be used for DNA extraction.

Suspension Cell Cultures: Cells (use ≤ 1 × 10^6 cells) are directly collected into a 1.5 mL tube. Centrifuge the cells at 2000 rpm for 3 minutes and discard the supernatant. Wash cells with 1 mL of PBS once by centrifugation at 2000 rpm for 3 minutes. The supernatant should be discarded and the precipitated cells will be used for DNA extraction.

Bodily Fluids: Up to 200 μL of bodily fluids including serum, plasma, or other body fluids like saliva can be processed. Fresh samples of bodily fluids are recommended. Frozen samples may be used; however, the yield of genomic DNA may be decreased.

Swabs:

a. By using sterile techniques, add 1.0 ml PBS to the cervical epithelial swabs container and vortex vigorously for 5 seconds. Remove the swab.

b. Transfer 500µl of the swab PBS to a new tube and centrifuge at 14,000 rpm for 3 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

c. Add 1.0 ml saline and resuspend the pellet by vortexing for 5 seconds. Centrifuge at 14,000 rpm for 3 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.

LBC (liquid-based cytology): HPV is a cell-associated virus, and a cellular sample from the site of infection is required.

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a. Transfer 500 µl about 10^4– 10^5cells from liquid-based medium (e.g. B9853) to the 1.5 ml tube containing 500 µl PBS and vortex vigorously for 5 seconds.

b. Centrifuge at 14,000 rpm for 1 minute. Carefully remove and discard supernatant from the tube without disturbing the pellet.

c. Add 1.0 ml PBS and resuspend the pellet by vortexing for 5 seconds. Centrifuge at 14,000 rpm for 1 minute. Carefully remove and discard supernatant from the tube without disturbing the pellet.

Elution procedures

Elution may also be performed with Tris-EDTA-buffer (TE) of pH equal to or higher than 8. This will increase DNA stability, especially during long-term and/or multi-use storage at 4 °C or ambient temperature by inhibition of omnipresent DNases. However, EDTA interferes, depending on the final concentration, with certain downstream applications. For optimal performance of isolated DNA in subsequent downstream applications, we recommend elution with the supplied elution buffer and storage, especially long term, at -20 °C. Several freeze-thaw cycles will not interfere with most downstream applications.

Convenient elution: For convenience, an elution buffer of ambient temperature may be used. This will result in a lower yield (approximately 20 %) compared to elution with preheated elution buffer.

High yield: Perform two elution steps with the volume indicated in the individual protocol. About 90–100 % of bound nucleic acid can be eluted.

High concentration: Perform one elution step with 60 % of the volume indicated in the individual protocol. The concentration of DNA will be higher than with standard elution.

High yield and high concentration: Apply half the volume of elution buffer as indicated in the individual protocol; incubate for 3 min at 60° C and centrifuge. Apply a second aliquot of elution buffer, incubate and

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centrifuge again. Thus, about 85–90 % of bound nucleic acid is eluted in the standard elution volume at a high concentration.

Kit Components

No.	Name	cat #: DB9822-50rxn	cat #: DB9822-100 rxn
1	*Handbook protocol*	1	1
2	Columns and Collection Tubes (pcs)	50	100
3	Collection Tubes (pcs)	100	200
4	1.5-2 ml Vials for sample (pcs)	50 pcs	100 pcs
5	L1 Buffer (Lysis Buffer)	11 ml	22 mL
6	Proteinase K* (Lyophilized)	As needed	As needed
7	Proteinase K buffer*	As needed	As needed
8	VB2 Solution	17 ml	33 ml
9	WB1	3 7 ml	75 ml
10	WB2	3 7 ml	75 ml
11	Elution buffer (EB)	5 ml	10 ml

*Proteinase K buffer should be mixed with lyophilized proteinase K and then stored at – 20° C for up to 18 months.

Note: During storage, especially at low temperatures, a precipitate may form in some Buffers. Such precipitates can be easily dissolved by incubating the bottle at 60 °C.

Storage condition:

Shipping: RT

Storage: The reconstituted proteinase K should be stored at – 20° C. All other kit components can be stored at room temperature (18–25 °C) and are stable for up to one year.

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Protocols of DNA Extraction

Before experiment notes:

* Check if Washing Buffers and proteinase K be prepared according to the procedure.

*Set an incubator, thermo block, or water bath to 65 °C.

*Preheat Elution Buffer BE to 65 °C.

* Centrifuge speed: 6000- 14000 *x g*

* Solve the PBS powder into 500 ml of DW (no need for pH adjustment).

A. Lysis step

1. Shake the LBC bottle (sample) and transfer 1 ml of LBC into a sample vial (provided) and centrifuge it at 10000-12000 *x g* for 2-4 min, discard 800 µl of the supernatant and keep 200 µl of it.
2. Add 20 µl proteinase K into the sample vial.

Then, Add 200 µl, L1 Buffer. **Mix thoroughly** by vortexing for 15 seconds, and incubate at 56°C for 15 min. **Vigorous vortexing** should be repeated **every 5 minutes**. Spin down briefly to remove any drops from the inside of the lid.

Note 1: Vigorous mixing is important to obtain a high yield and purity of DNA.
Note 2: Incubation times may fluctuate between 15 minutes to over 2 hours depending on the type of tissue being lysed. Lysis is considered complete when a relatively clear lysate is obtained.

B. Adjust DNA binding conditions

3. Add 300 µl of VB2 Solution to the sample, and mix by inverting. Incubate for 1 min at room temperature. Spin down briefly to remove any drops from the inside of the lid.

C. DNA binding

4. Transfer the mixture from step 4 into the column placed in a 2 ml collection tube. Centrifuge for 1 min at 8000 *x g*. Discard flow-through.
   Note: For genital warts, a small part of the tissues should not be transferred to the column.

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D. Wash silica membrane

5. Place the column into a new collection tube, add 700 µl Wash Buffer1, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.
6. Place the column in a new 2 ml collection tube, add 700 µl Wash Buffer2, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.

E. Dry silica membrane

1. Place the column into the collection tube, then centrifuge at full speed (>12,000 *x g*) for 3 min to dry the membrane completely. Discard flow-through and collection tube. Residual ethanol is removed during this step.

   Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.

F. Elute highly pure DNA

7. Place the column into a sterilized 1.5 ml microcentrifuge tube and apply 40-60 µl (according to the detection kit, which should be set up by the operator for best result) Elution Buffer to **the center of the membrane and wait for 1 min**.
8. Close the lid and centrifuge at **12000 *x g*** for 1 min.

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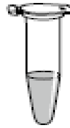
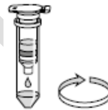
Procedure at a glance:

Steps		
1 Lyse samples		200 μL Bodily Fluid, cells, and tissue 200 μL L1 20 μL Pro. K Mix About 15 min at 65 °C
2 Adjust DNA binding conditions		300 μL VB2
3 Bind DNA		Load all 1 min 8,000 x *g*
4 Wash silica membrane 1st: 600 ul WB1 2nd: 600 ul WB2		Load all 1 min 8,000 x *g*
5 Dry silica membrane		3 min 12000 x *g*
6 Elute highly pure DNA		40-50 ul EB 65° C 1-2 min 12000 x *g* 1min

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Troubleshooting

Problem	Possible cause	Suggestions
The yield of genomic DNA is low	Incomplete lysis of cells	Extend the incubation time of Lysis or change the sample and use a fresh one.
	The DNA elution is incomplete	Ensure that centrifugation at max speed is performed, to ensure that all the DNA is eluted. Take care about step 7 in the protocol.
	Old sample	Fresh samples are recommended for maximum genomic DNA yield
	Inappropriate LBC	You should use standard LBC
No results in PCR	The template concentration must be optimized according to each detection kit.	

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