DNAbioTech
Life Science

DNAbiotech
Biotechnology is our expertise

DNA Extraction Kit

Liquid-based cytology media and Tissue

(Specifically for HPV)

Catalog no.: DB9822

(50 and 100 prep)

Intended for research use only

Modares Technology and Sciences Park, Room 510. No. 15, Gordafarid-heyat junction, North Kargar, Enghelan Square, Tehran, I.R. Iran

Www.dnabiotch.ir

Diba Noavaran Azma Company

Customer and technical support

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

About this user manual

It is recommended for the 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.

Contact us at
www.dnabiotech.ir
E-mail: dnabiotechco@gmail.com

Note: .....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....

Contents

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 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 20–40 ng per µL.

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

Warranty: the 1st reaction of this product may be used as free sample and if desired results do not obtained 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**.**

Safety Notes

The buffers included in "**DNAbiotech DB9822 DNA extraction kit"** contain irritant which 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 protector, and follow standard safety precautions.

Buffer L1 contain chaotropes 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 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 unsectioned core. Trim off any excess paraffin. Grind the sample into fine powder using **liquid nitrogen**.

b. Transfer the sections or ground block into a Nuclease-free microcentrifuge 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 Step **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.

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. 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. 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.

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

b. Centrifuge at 14,000 rpm for 1 minutes. 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 minutes. 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 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, elution buffer of ambient temperature maybe 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. 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

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	Extra Collection Tubes	100	200
4	L1 Buffer	10 ml	20 ml
5	Proteniase K* (Lyophilized)	As needed	As needed
6	Proteniase K buffer*	As needed	As needed
7	WB1 (Concentrate)**	33 ml (22 ml ethanol should be added)	2 x 33 ml (2 x 22 ml ethanol should be added)
8	WB2 (Concentrate)**	11ml (44 ml ethanol should be added)	2 x 11 ml (2 x 44 ml ethanol should be added)
9	Elution buffer (EB)	10 ml	20 ml
10	PBS powder for 500 ml***	1 aluminum sache	1 aluminum sache

*Proteniase K buffer should be mix with lyophilized proteniase K and then stored at – 20°C for up to 18 months.

** 96-100 % pure ethanol should be added.

*** Solve the PBS powder into 500 ml of DW (no need to pH adjustment). It can be stylized by autoclave of 0.22 micrometer filtration (optimal).

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 up to one year.

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 to pH adjustment).

A. Lysis step

1. Preparation of samples:

a. For Bodily Fluid: As indicated in the "sample preparation part".
b. For Swabs: As indicated in the "sample preparation part".
c. For cells: about 10^5-10^6 precipitated cells (As indicated in the "sample preparation part")
d. For LBM: about 10^4-10^5 precipitated cells (As indicated in the "sample preparation part")
e. For Tissue (genital warts) and FFPE: As indicated in the "sample preparation part".

2. Add 200 µl of L1 Buffer to the cell pellet. Mix by vigorous vortexing

3. Add 20 µl proteinase K to the sample, **mix thoroughly** by vortexing, and incubate at 65°C for 15 min. Spin down briefly to remove any drops from inside of the lid.

Note 1: Vigorous mixing is important to obtain high yield and purity of DNA.

Note 2: Incubation times may fluctuate between 45 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

4. Add 200 µl of absolute ethanol to the sample, and mix thoroughly by vortexing. Incubate for 1 min at room temperature. Spin down briefly to remove any drops from inside of the lid.

C. DNA binding

5. Transfer the mixture from step 4 into the column placed in a 2 ml collection tube. Centrifuge for 1 min at 6000 *x g*. Discard flow-through.
   Note: For genital warts, small part of the tissues hould not be transferred to the column.

D. Wash silica membrane

6. Place the column into a new collection tube, add 600 µl Wash Buffer1, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.
7. Place the column in a new 2 ml collection tube, add 600 µl Wash Buffer2, and centrifuge for 1 min at 8000 *x g*. Discard flow-through.

E. Dry silica membrane

8. Place the column into the collection tube, then centrifuge at full speed (>12,000 *x g*) for 2-3 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

9. Place the column in a clean 1.5 ml microcentrifuge tube. Carefully open the lid of the column and apply 100-200 µl prewarmed Elution Buffer to the center of the membrane.
10. Close the lid and incubate at 65° C for 1-2 min. Centrifuge at full speed (>12,000 *x g*) for 1 min.

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		200 µL ethanol
3 Bind DNA		Load all 1 min 6,000 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		2-3 min 14000 x *g*
6 Elute highly pure DNA		100-200 ul EB 65° C 1-2 min 14000 x *g* 1min

Troubleshooting

Problem	Possible cause	suggestions
The spin column is clogged	*The sample is too large*	Do not exceed the recommended amount of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. Clogging can also be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
The lysate is very gelatinous prior to loading onto the column	*The lysate/binding solution mixture is not homogeneous*	To ensure a homogeneous solution, vortex for 10-15 seconds before applying the lysate to the spin column.
	Maximum number of cells or amount of tissue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
The yield of genomic DNA is low	*Improper storage of samples*	Tissue samples and cell pellets may be frozen and stored at -20°C or -70°C. Repeated freezing and thawing of stored samples should be avoided, as this may lead to decreased yields of DNA.
	Incomplete lysis of cells	Extend the incubation time of proteinase K digestion or reduce the amount of tissue or cells used for lysis.
	The DNA elution is incomplete	Ensure that centrifugation at 14,000 x g is performed, to ensure that all the DNA is eluted.
The genomic DNA is sheared	*The genomic DNA was handled improperly*	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).
	Improper storage of sample	Repeated freezing and thawing of stored samples should be avoided as this may lead to decreased DNA size.
	The sample is old	Sheared DNA may be obtained from old tissue or cell samples. Fresh samples are recommended for maximum genomic DNA yield
	Carry-over of	Be sure to remove all of ethanolic Buffer

	ethanol	before eluting the DNA. If the level of WB after the second wash has reached the column outlet for any reason, discard flow-through, place the column back into the Collection Tube, and centrifuge again.
Suboptimal Performance of genomic DNA in enzymatic reactions	*Contamination of DNA with inhibitory substances*	If DNA has been eluted with Tris / EDTA-buffer (TE), make sure that EDTA does not interfere with downstream applications or repurify DNA and elute in Buffer BE. If the A260 / A280 ratio of the eluate is below 1.6, repeat the purification procedure: Add 1 volume of Buffer WB 3 plus 1 volume ethanol to the eluate, load on spin column and centrifuge for 1 min at 10000 x g.
No band in PCR	*Optimize the concentration of template*	If the template be less or more than usual, PCR reaction won't be able to amplify the DNA target. Use a nanodrop and find the exact concentration of the DNA and apply the template as it is indicated in your PCR procedure.

Note: .....
.....
.....
.....
.....
.....
.....
.....
.....
.....
.....

Related products:	***Some other products***
LBC kit (Liquid based cytology)	SDS-PAGE preparation kit
50 X TAE buffer	Bradford Protein assay kit
10 X TBE buffer	MTT Assay kit
5 X TBE buffer	TMB ELISA kit (3 reagents)
Ready to use PBS buffer for 500 ml and 1 liter	BCA kit (different reaction and volumes)
ECO DNA safe stain for gel (gel stain)	Ethidium bromide dropper 5ml, 10 mgr / ml solution, molecular
Load safe DNA stain (for sample)	Gel buffer stacking gel
6 X DNA loading buffer	Gel buffer Separation
Agarose (10, 25, 50 and 100 gr)	Sample bufferSDS-PAGE
DNA extraction kit from whole blood	Acryl amid/bis acryl Amid Stock Solution (30/0.8 %)
DNA extraction kit from tissue	DEPC treated water
Plasmid DNA extraction kit	PMSF
DNA extraction kit from bacteria	IPTG
DNA extraction kit from stool	RIPA buffer
Total DNA extraction kit	TrizolEX (25,50 and 100 ml)
Total RNA extraction kit	Saturated phenol
DNA extraction kit from plant	Gram staining kit
Spin columns (different types)	Diff quick kit
Collection tube	Trypcin-EDTA 0.25 100 ml
RNase A solution (10 mgr/ml)	100 X L glutamine Solution 100 ml
Proteinase K solution (20 mgr/ml)	Penicillin-Streptomycin 100X
Absolute Ethanol	DMSO for cell stock
Isopropanol (2-propanol)	DMSO for MTT assay
Taq DNA polymerase master mix	RNA stabilization solution
PFU master mix	LB agar and LB broth media
And….	*And….*

DNAbiotech is the 1st Iranian Biotechnology Company producing wide ranges of ready to use **molecular buffers**. These products are produced from the high quality ingredients and help researchers run their projects faster.

Other products & services:

- ✓ Cloning and expression of different recombinant peptides
- ✓ Gene, Primer and peptide synthesizing
- ✓ Column based DNA extraction kits from different samples
- ✓ RNA extraction kit
- ✓ Bioinformatics services
- ✓ Production of secondary antibodies (goat anti mouse, anti rabbit and anti human antibodies, HRP conjugated).
- ✓ Taq polymerase and PFU master mix
- ✓ Molecular grade buffers (TAE, TBE, RIPA and….)
- ✓ Column based DNA/RNA extraction kits.
- ✓ And ….

For more information visit us at "www.dnabiotech.ir"

More Products Launch Coming Soon!