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Buccal Swab DNA Purification Kit

Rev 06/21

(Catalog # K1466-50, -250; 50 or 250 Preps; Store at Multiple Temperatures)

I. Introduction:

BioVision's Buccal Swab DNA Purification Kit provides a rapid and easy method for isolating genomic DNA from forensic samples including Buccal Swabs using spin columns. In this kit, the samples are first lysed and applied to the spin column. The DNA binds to the spin column, while cellular debris and other proteins are effectively washed away by DNA Wash Buffer. Pure DNA is then eluted using sterile deionized water or elution buffer. Each spin column can bind approximately 100 µg DNA. This kit does not require phenol/chloroform extraction or isopropanol/ethanol precipitation. DNA purified using this kit is ready for downstream applications such as PCR, Southern blotting, restriction digestion etc.

II. Application:

- To extract genomic DNA from Buccal Swabs

III. Key Features:

- Rapid, easy and convenient
- **Highly pure**, high yield
- **Many downstream applications** such as PCR, Southern blotting etc.
- High quality spin columns

IV. Sample Types:

- Buccal Swabs

V. Kit Contents:

Components	K1466-50 (50 Rxns)	K1466-250 (250 Rxns)	Part Number
Buffer BL	15 ml	75 ml	K1466-XX-1
Protease K	1.5 ml	6.5 ml	K1466-XX-2
DNA Columns	50	250	K1466-XX-3
*DNA Wash Buffer	12 ml	50 ml	K1466-XX-4
Elution Buffer	10 ml	25 ml	K1466-XX-5

DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1466-50) or 200 ml (K1466-250) 100% Ethanol to DNA Wash Buffer bottle before use. Be sure to close the bottle tightly after each use to avoid Ethanol evaporation.

VI. User Supplied Reagents and Equipment:

- Pipettes
- Pipette tips
- 100% Ethanol
- DD Water
- PBS
- Sterile, nuclease-free 1.5 and 2 ml microcentrifuge tubes
- Microcentrifuge

VII. Shipping and Storage Conditions:

The kit is shipped in a gel pack. All reagents except Protease K must be stored at room temperature (RT). Protease K must be stored at -20°C. The kit reagents are stable for 12 months if stored properly.

VIII. Reagent Preparation and Storage Conditions:

1. DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1466-50) or 200 ml (K1466-250) 100% Ethanol to DNA Wash Buffer bottle before use.
2. Buffer BL contains acid and chaotropic salts, which may form reactive compounds with bleach. Do not add bleach or acidic solutions directly to the preparation waste. Wear gloves and protective eyewear when handling this buffer.
3. A precipitate may form in Buffer BL under cool ambient conditions. Warm the bottle at 37°C to dissolve the precipitate before use.

IX. Swab DNA Purification Protocol:

This protocol yields typically about 0.5-3 µg DNA from cotton or C.E.P. swabs.

1. Follow the standard protocol for **obtaining swab samples**. Scrape the swabs firmly against the inside of the cheek 5-10 times. Air-dry or vacuum the swabs for 1-2 hours after collection. The person providing the sample should not eat or drink for at least 30 min prior to the sample collection.
2. Remove, transfer and **add the buccal swab** into a 2 ml microcentrifuge tube.
3. Add 280 µl **PBS**, 25 µl **Protease K** and 280 µl **Buffer BL** to the sample. Mix immediately by vortexing for 30 sec. Incubate for 30 min at 50°C with occasional mixing. Briefly centrifuge to remove any droplets from inside the lid.
4. Add 525 µl **100% ethanol** and mix thoroughly by vortexing. Briefly centrifuge to collect any droplets from the lid.
5. Insert a **DNA column** into a 2.0 ml microcentrifuge tube. Carefully apply 600 µl of the mixture into the column. Centrifuge at 10,000 rpm for 30 sec. Discard the flow-through liquid.

6. Carefully apply the remaining mixture to the column and centrifuge at 10,000 rpm for 30s. Discard the flow-through liquid.
7. Place the column in a microcentrifuge tube. Add 600 µl of **DNA Wash Buffer** and centrifuge at 10,000 rpm for 1 min. Discard the flow-through liquid.
8. Add 600 µl of **DNA Wash Buffer** and centrifuge at 10,000 rpm for 1 min. Discard the flow-through liquid. Place the column with the lid open in a microcentrifuge tube.
9. Centrifuge at 13,000 rpm for 2 min to dry the column. This step is critical for the removal of residual ethanol that might cause a decrease in yield and purity of DNA.
10. Place the column in a sterile nuclease-free 1.5 ml microcentrifuge tube and add 100 µl of preheated (70°C) **Elution Buffer**. Incubate the tube at 70°C for 3 min.
11. Centrifuge at 10,000 rpm for 1 min to **elute the DNA**.

Note: Adding the eluted DNA back to the column for a second elution will yield another 20% of bound DNA. Incubation at 70°C rather than at RT will give a modest increase in the DNA yield.

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X. General Troubleshooting Guide:

Problem	Possible Reason	Suggested Improvement
Colored residue in column after washing	Forgot to add ethanol	Before applying sample to column, both Buffer BL and ethanol must be added.
	Forgot to add ethanol to DNA Wash	Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use.
	Incomplete lysis due to improper mixing	Buffer BL is viscous and the sample must be vortexed thoroughly.
Column clogged	Sample is too viscous	Divide the sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl.
Low DNA yield	Clogged column	See above.
	Poor elution	Repeat elution or increase the elution volume. Incubating the column at 70°C for 5 min with Elution Buffer may increase the yield.
	Improper washing	DNA Wash Buffer Concentrate must be diluted with 100% ethanol as specified before use.
A_{260}/A_{280} ratio lower than 1.7	Extended centrifugation during elution step.	Resin from the column may be present in the eluate. Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation. It will not interfere with PCR or restriction digests.
	Poor cell lysis due to incomplete mixing with Buffer BL	Repeat the procedure. Make sure to vortex the sample with Buffer BL immediately and completely.
	Samples are rich in protein	After applying the sample to DNA Column, wash with 300 µl of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.

XI. Related Products:

BioVision Product Name	Cat. No.	Sizes
Dried Body Fluids DNA Purification Kit	K1464-50, -250	50, 250 Preps
Sperm DNA Purification Kit	K1465-50, -250	50, 250 Preps
Whole Blood DNA Isolation Kit	K528	100 Preps
Mammalian Cell Genomic DNA Isolation Kit	K967	100 Preps
Soil Genomic DNA Kit	K1411-50, -250	50, 250 Preps
Yeast Genomic DNA Kit	K1414-50, -250	50, 250 Preps
Fungal Genomic DNA Kit	K1415-50, -250	50, 250 Preps
96-well Viral DNA/RNA Kit	K1417-1, -4	1, 4 Plates
Mitochondrial DNA Isolation Kit	K280	50 Preps
Blood genomic DNA extraction and purification kit	K1443	100 Preps

FOR RESEARCH USE ONLY! Not to be used on humans.