Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Eye, Nose & Swabs gDNA Purification Kit

04/20

(Catalog # K1467-50, -250; 50 or 250 Preps; Store at MT)

I. Introduction:

BioVision's Forensic Genomic DNA Purification Kit provides a rapid and easy method for isolating genomic DNA from forensic samples including eye, nose and 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 gDNA from Eye, Nose and 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:

- Eye, Nose and other Swabs

V. Kit Contents:

Components	K1467-50 (50 Rxns)	K1467-250 (250 Rxns)	Part Number
Buffer TL	15 ml	75 ml	K1467-XX-1
Protease K	1.5 ml	6.5 ml	K1467-XX-2
Buffer BL	15 ml	75 ml	K1467-XX-3
DNA Columns	50	250	K1467-XX-4
Buffer KB	12 ml	50 ml	K1467-XX-5
*DNA Wash Buffer	12 ml	50 ml	K1467-XX-6
Elution Buffer	10 ml	25 ml	K1467-XX-7

DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1467-50) or 200 ml (K1467-250) 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 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 will be 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 (K1467-50) or 200 ml (K1467-250) 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. Genomic DNA Purification Protocol:

1. Collect the **sample** and add 2 ml **PBS**. Incubate for 2-3 h at 30°C.
2. Pellet the sample by centrifuging at 8, 000 rpm for 10 min.
3. Resuspend the pellet in 200 µl **Buffer TL**.
4. Add 25 µl **Protease K** and mix well by votexing. Incubate at 50°C for 30-60 min with occasional mixing. Spin the sample briefly to collect droplets from the cap.
5. Add 225 µl **Buffer BL** and mix well by vortexing. Incubate at 60°C for 10 min. Spin the sample briefly to collect droplets from the cap.
6. Add 225 µl **100% Ethanol** and mix thoroughly by vortexing. Briefly centrifuge to remove any droplets inside the tube.

7. Place a **DNA Column** into a microcentrifuge tube. Apply the entire sample into the DNA column, including any precipitate that may have formed. Centrifuge at 10,000 rpm for 1 min. Discard the flow-through liquid.
8. Transfer the DNA Column into the microcentrifuge tube and add 500 µl of **Buffer KB** into the column. Centrifuge at 10,000 rpm for 30 sec. Discard the flow-through liquid.
9. Add 600 µl **DNA Wash Buffer**. Centrifuge at 10,000 rpm for 30 sec. Discard the flow-through liquid.
10. Add 600 µl **DNA Wash Buffer** and centrifuge at 10,000 rpm for 30 sec. Discard the flow-through and place the DNA column with the lid open in the microcentrifuge tube.
11. Centrifuge the DNA Column at 13,000 rpm to dry the column. This step is critical for removing the residual ethanol that might interfere with the yield and purity of DNA.
12. Place the column in a sterile nuclease-free 1.5 ml microcentrifuge tube (not provided) and add 100 µl of pre-warmed (70°C) **Elution Buffer**. Incubate the tube at 70°C for 3 min.
13. **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 DNA yield per elution.*

X. General Troubleshooting Guide:

Problem	Possible Reason	Suggested Improvement
Colored residue in column after washing	Forgot to add ethanol	Before applying sample to the column, both Buffer BL and ethanol must be added.
	Forgot to add ethanol to DNA Wash Buffer	Dilute DNA Wash Buffer with the indicated volume of absolute ethanol before use.
	Incomplete lysis due to improper mixing with Buffer BL	Buffer BL is viscous and the sample must be vortexed thoroughly.
Column clogged	Incomplete lysis	Extend incubation time of lysis with Buffer TL and protease. Add the correct volume of Buffer BL and incubate for specified time at 70°C. It may be necessary to extend the incubation time by 10 min.
	Sample is too large	If using more than 30 mg sample, increase the volume of Proteinase K, Buffer TL, Buffer BL, and ethanol. Pass aliquots of lysate through one column successively.
	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.
	Incomplete cell lysis or protein degradation due to insufficient incubation.	Increase incubation time with Buffer TL and Protease K. Ensure that no visible pieces of sample remain.
	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
Buccal Swab DNA Purification Kit	K1466-50, -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

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