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Sperm DNA Purification Kit

04/20

(Catalog # K1465-50, -250; 50 or 250 Preps; Store at Multiple Temperatures)

I. Introduction:

BioVision's Sperm DNA Purification Kit provides a rapid and easy method for isolating genomic DNA from forensic samples including Sperm 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 Sperm genomic DNA from semen samples

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:

- Fresh or frozen semen

V. Kit Contents:

Components	K1465-50 (50 Rxns)	K1465-250 (250 Rxns)	Part Number
10X Buffer A	50 ml	250 ml	K1465-XX-1
Buffer B	15 ml	70 ml	K1465-XX-2
Protease K	1.5 ml	6.5 ml	K1465-XX-3
Buffer BL	15 ml	75 ml	K1465-XX-4
DNA Columns	50	250	K1465-XX-5
Buffer KB	12 ml	50 ml	K1465-XX-6
*DNA Wash Buffer	12 ml	50 ml	K1465-XX-7
Elution Buffer	10 ml	25 ml	K1465-XX-8

DNA Wash Buffer must be diluted with 100% Ethanol before starting. Add 48 ml (K1465-50) or 200 ml (K1465-250) 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
- Glass (Corex) centrifuge tubes
- β-mercaptoethanol
- 100% Ethanol
- DD Water
- Sterile, nuclease-free 1.5 ml microcentrifuge tubes
- Microcentrifuge and Centrifuge

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 (K1465-50) or 200 ml (K1465-250) 100% Ethanol to DNA Wash Buffer bottle before use.
2. Add DD Water to prepare 1X Buffer A from 10X Buffer A before use.
3. Add β-mercaptoethanol to a final percentage of 2% (v/v) to Buffer B.
4. 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.
5. A precipitate may form in Buffer BL under cool ambient conditions. Warm the bottle at 37°C to dissolve the precipitate before use.

IX. Sperm DNA Purification Protocol:

This protocol can be used for fresh or frozen semen samples with equal efficiency. Frozen samples must to be thawed thoroughly before use. The time for lysis will vary depending on the size and density of the source material.

1. Add **50-250 µl** of Semen samples to 10 ml of **1X Buffer A** in a glass (Corex) centrifuge tube. Vortex for 10 sec at full speed. Use only Corex tubes to prevent attachment of the sperm cells to the tube walls.

2. Centrifuge for 10 min at 4,000 rpm (2500 x g).
3. Carefully remove the supernatant leaving 1 ml of pellet and **1X Buffer A**.
4. Vortex for 5 sec and centrifuge at full speed to collect any sample adhering to the walls of tubes. Transfer the entire sample to a microcentrifuge tube.
5. Add 0.5 ml of **1X Buffer A** to the Corex tube and vortex for 30 sec and centrifuge at full speed to collect any sample adhering to the walls of the tube. Transfer the sample to the microcentrifuge tube in step 4. Centrifuge for 2 min at full speed.
6. Carefully remove the supernatant without disturbing the semen pellet.
7. Resuspend the pellet in 200 µl of **Buffer B containing β-mercaptoethanol** to a final percentage of 2% (v/v).
8. Add 25 µl of **Protease K** and incubate for 2 hr at 50°C.
9. Invert the tube occasionally to disperse the sample or place on a rocking platform.
10. Add 250 µl **Buffer BL** and 260 µl **100% Ethanol** to the sample and mix by vortexing.
11. 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.
12. 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.
13. Add 600 µl **DNA Wash Buffer**. Centrifuge at 10,000 rpm for 30 sec. Discard the flow-through liquid.
14. 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.
15. 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.
16. 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.
17. **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 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	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
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.