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Mammalian Cell Genomic DNA Isolation Kit

07/16

(Catalog # K967-100; 100 isolations; Store at Multiple Temperatures)

I. Introduction:

Isolation of high quality DNA from mammalian cells and tissue in sufficient quantities is important for many basic research and clinical applications. BioVision's Mammalian Cell Genomic DNA Isolation Kit facilitates purification of quality DNA from mammalian cultured cells or tissue. This kit utilizes enzymatic reactions to release genomic DNA from the cells. Upon release, DNA is adsorbed onto a silica spin-column under chaotropic conditions, eliminating the use of toxic organic compounds or solvents. DNA purified by this kit is suitable for various downstream molecular biology applications such as PCR, cloning, DNA hybridization, Southern Blotting, sequencing, genotyping, enzymatic analysis, and more.

II. Applications:

- PCR, cloning, DNA hybridization, southern blotting, genotyping, restriction enzyme digest analysis, sequencing, etc.

III. Sample Type:

- Mammalian cultured cells or tissue

IV. Kit Contents:

Components	K967-100	Cap Code	Part Number	Storage (°C)
Buffer R [Re-suspension Buffer]	25 ml	NM/Clear	K967-100-1	RT
Enzyme Mix	1.5 ml	Red	K967-100-2	-20°C
RNAse A	1.5 ml	Blue	K967-100-3	-20°C
Buffer B [Binding Buffer]	25 ml	NM/Clear	K967-100-4	RT
Buffer W [Wash Buffer]	30 ml	WM	K967-100-5	RT
Buffer E [Elution Buffer]	20 ml	WM	K967-100-6	RT
Spin Columns/Collection Tubes	100 tubes	-	K967-100-7	RT

V. User Supplied Reagents and Equipment:

- DNAse-free aerosol tips and micro-centrifuge tubes, 100% Ethanol, Heating Block, Centrifuge

VI. Storage Conditions and Reagent Preparation:

Refer to section IV (Kit contents) for proper storage. Protect from light. Briefly centrifuge small vials prior opening. Read entire protocol before performing the assay.

- **Buffer R and Buffer E:** Ready to use. Store at room temperature.
- **Buffer B:** Add 28 ml of 100% Ethanol, molecular biology grade. Mix well and store at room temperature.
- **Buffer W:** Add 132 ml of 100% Ethanol, molecular biology grade. Mix well and store at room temperature.
- **Enzyme Mix and RNAse A:** Ready to use. Store at -20°C. Keep on ice at all times while in use.
- **Spin Columns/Collection Tubes:** Ready to use. Store at room temperature in dry conditions.

VII. Whole Blood DNA Isolation Protocol:

1. Sample Preparation – Cell Lysis:

a. Count cells and aliquot $0.25X10^6$ – $2.5X10^6$ cells into a 1.5 ml microfuge tube or weigh out 20 mg of tissue previously disrupted in liquid nitrogen with mortar and pestle.
Note: *If using > $2.5X10^6$ cells or > 20 mg of tissue, scale up the entire protocol proportionally.*

b. To prepare cultured cells for DNA Isolation:
- **i.** Centrifuge cells at 2000 x g for 5 minutes at 4°C and discard the supernatant.
- **ii.** Thoroughly re-suspend the cell pellet in 1 ml of PBS to wash and centrifuge at 2000 x g for 5 minutes at 4°C.
- **iii.** Discard the supernatant and move immediately to "DNA Release" section.

2. DNA Release:

c. Re-suspend mammalian cells or disrupted tissue in 250 µl Buffer R [Re-suspension Buffer].

d. *Optional: Add 15 µl of RNAse A at this point if you wish to obtain RNA-free DNA product*.

e. Add 15 µl of Enzyme Mix and mix by inverting the tube 5 times. Incubate for 30 minutes at 55°C.

Note: *Mammalian tissue sample may have leftover cellular debris at this point. To prevent column clogging, centrifuge the tubes for 1 minute on max and use the supernatant in the next step; discard the pellet.*

3. Binding:

f. Add 500 µl of Buffer B [Binding Buffer] and mix by pipetting up and down until the solution becomes homogenous.

g. Place the spin column into the collection tube (both provided with the kit) and pipette the entire supernatant onto the top of the column.

h. Centrifuge the spin column at 12,000 x g for 1 minute at 4°C and discard the flow through.

4. Washing:

i. Add 750 µl of Buffer W [Wash Buffer] onto the top of the spin column and centrifuge at 12,000 x g for 1 minute at 4°C. Discard the flow through.

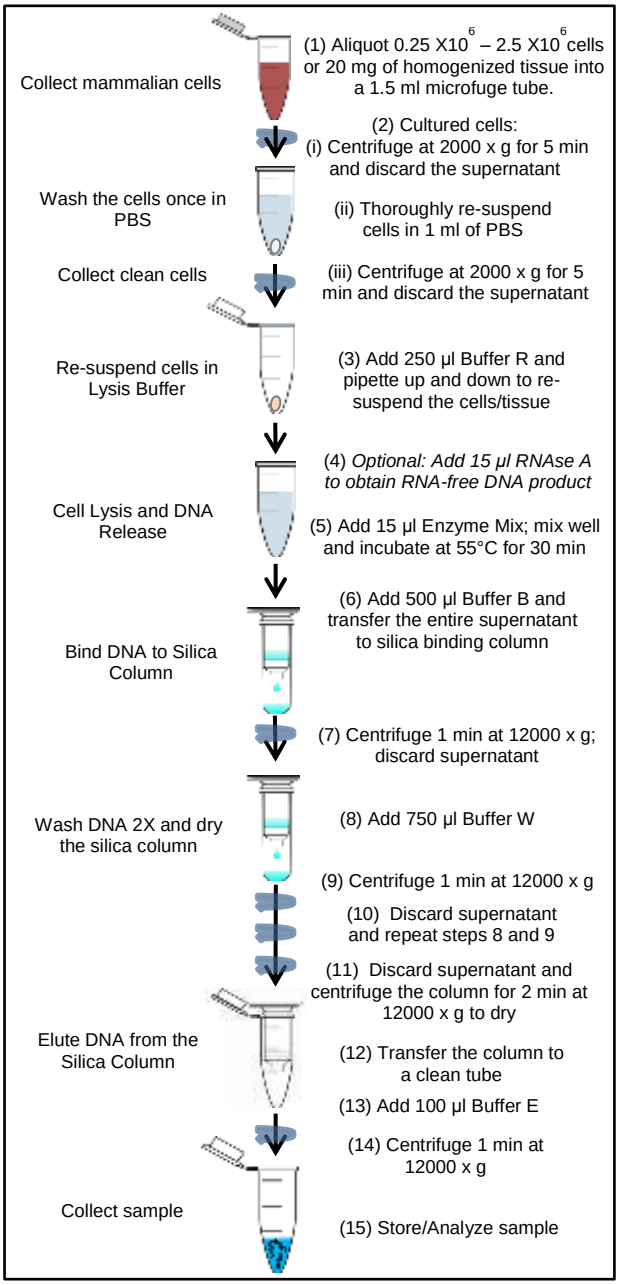
j. Repeat step "i" one more time.

k. Centrifuge the spin column at 12,000 x g for 2 minutes at 4°C to dry.

5. Elution:

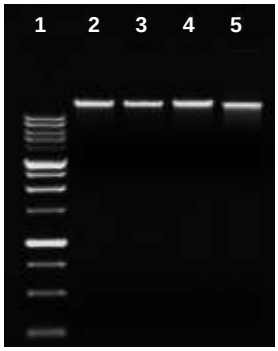
l. Transfer the spin column to a clean, DNAse-free 1.5 ml tube.
m. Add 100 µl of Buffer E [Elution Buffer] to the top of the spin column and incubate for 1-2 minutes at RT.
n. Centrifuge at 12,000 x g for 1 minute at 4°C. The flow through contains purified DNA.
o. Store DNA at -20°C or immediately use the sample in a downstream application of your choice.
Note: *Good quality DNA will have A260/280 of 1.7- 1.99 and exhibit one clear band of high molecular weight on 1% agarose gel. See troubleshooting guide in Section IX for help.*

VIII. Protocol Quick Guide



IX. Trouble Shooting

Issue	Possible Reason	Recommendations
Low yield	The tissue is not fresh / the cells are not viable	Use fresh tissue / ensure that the cultured cells are viable prior to their lysis.
	Incomplete DNA release	Increase incubation with Enzyme Mix up to 45min – 1hr.
Low A260/280 (<1.7)	Protein Contamination	Increase incubation time with Enzyme Mix up to 45min – 1hr.
High A260/280 (>2.0)	RNA Contamination	Add RNAse A during DNA release reaction step "d"
No DNA band/smear	DNAse contamination	Use DNAse free aerosol tips, DNAse-free tubes, and practice good sterile technique.
Clogged Column	Incomplete disruption of tissue / contaminants present	Use a smaller amount of cells or tissue free of fat and other contaminants.



1% Agarose – TBE gel

Figure 1: Example Genomic DNA Isolation

Gel Lane Description:
BioVision's BriteRuler 1kb DNA Ladder (cat# 9301-100) – Lane 1; Genomic DNA extracted from EA.hy 926 cells – Lane 2, HT 1080 cells – Lane 3, Jurkat cells – Lane 4, and rat kidney tissue – Lane 5.

FOR RESEARCH USE ONLY!
Not to be used on humans