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Gel and PCR DNA Purification Kit

02/20

(Catalog # K1455-50, -250; Store at RT)

I. Introduction:

BioVision's Gel and PCR DNA Purification Kit allows the purification of DNA from agarose gels, PCR, RFLP, phosphorylation, labeling and other enzymatic reactions. In this kit, DNA fragments bind to the mini columns and the DNA fragments are eluted in an elution buffer. The purified DNA is ready for downstream applications including Sequencing and Restriction Enzyme Digestion.

II. Application:

Purifying DNA from agarose gels, PCR, RFLP, phosphorylation, labeling and other enzymatic reactions

III. Key Features:

- High Efficiency: > 90% recovery of DNA
- Fast and Reliable
- All the centrifugation steps can be performed at room temperature
- Broad Fragment Size Range: 200 bp - 20 kb
- High quality spin columns

IV. Sample Types:

- DNA fragments from Agarose gels, Restriction Enzyme Digestion products, PCR, RFLP, phosphorylation, labeling and other enzymatic reactions

V. Kit Contents:

Components	K1455-50 (50 Preps)	K1455-250 (250 Preps)	Part Number
Buffer GC	28 ml	120 ml	K1455-XX-1
*DNA Wash Buffer	12 ml	50 ml	K1455-XX-2
Elution Buffer	10 ml	30 ml	K1455-XX-3
Mini Columns	50	250	K1455-XX-4
Collection tubes	50	250	K1455-XX-5

**DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 48 ml (K1455-50) or 200 ml (K1455-100) 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 and Pipette tips
- 100% ethanol
- DD water (RNAse/DNAse free)
- 1.5 ml microcentrifuge tubes
- Microcentrifuge
- Waterbath
- Vacuum manifold if using vacuum protocol

VII. Shipping and Storage Conditions:

All kits are shipped and stored at room temperature (RT). All reagents are stable for up to 12 months when stored properly at RT.

VIII. Reagent Preparation and Storage Conditions:

1. DNA Wash Buffer must be diluted with 100% ethanol before starting. Add 48 ml (K1455-50) or 200 ml (K1455-250) to Buffer GC bottle before use. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.
2. Buffer GC may form precipitates under cool ambient condition. Warm up the buffer at 37°C to dissolve before use.
3. If crystals form in wash or elution buffers, warm at 37°C to dissolve before use.
4. Pre-warm the Elution Buffer at 55°C.

IX. Gel Extraction and DNA Purification Protocol:

1. **For Agarose gel:** Excise the DNA fragment from the agarose gel and weigh it in a 1.5 ml microtube. A gel slice of 100 mg equals to a volume of 100 µl. Add **1 volume** of **Buffer GC** to the 1.5 ml microtube and incubate the mixture at 55-60°C for 8 min. Mix the tube by tapping the bottom every 2 min till the gel has melted completely. Cool the tube to RT.

   For PCR reactions: Add **1 volume** of **Buffer GC** to the PCR reaction and mix completely by vortexing. Briefly spin the tube to collect any drops from the inside wall and the tube lid.
2. Transfer up to **700 µl of the DNA/Buffer GC mixture** to a spin column with a collection tube. Centrifuge at 13,000 x g for 1 min at RT. Discard the flow-through and put the column back to the collection tube. Repeat this step to process the remaining solution.
3. **Optional step for extraction of DNA from gels**: Add **500 µl of Buffer GC** to the DNA Mini Column. Centrifuge at 13,000 x g for 1 min at RT. This step is helpful for blunt-end ligation and other very sensitive applications. Discard the flow-through and put the column back into the collection tube.

4. Add **500 µl of DNA Wash Buffer** (make sure ethanol is added to DNA Wash Buffer) to the column and centrifuge at 13,000 x g for 1 min at RT. Discard the flow through. Repeat this step.
5. Centrifuge the empty column, at 13,000 x g for 1 min to remove the residual ethanol in the column. This is critical for DNA yield.
6. Place the column in a clean 1.5 ml micocentrifuge tube and add **35-50 µl Elution Buffer** to the column. Incubate at RT for 1 min. Centrifuge at 13,000 x g for 1 min to elute the DNA. Pre-warming the elution buffer to 55°C will increase the DNA yield.
7. **Optional:** Re-apply the eluate to the column for another elution. This increases the DNA yield and concentration.

X. Vacuum/Spin Protocol for PCR/Gel Extraction

1. **For agarose gel:** Excise the DNA fragment from the agarose gel and weigh it in a 1.5 ml microtube. A gel slice of 100 mg equals to a volume of 100 µl. Add **1 volume** of **Buffer GC** to the 1.5 ml microtube and incubate the mixture at 55-60°C for 8 min. Mix the tube by tapping the bottom every 2 min till the gel has melted completely. Cool the tube to RT.

   For PCR reactions: Add **1 volume** of **Buffer GC** to the PCR reaction and mix completely by vortexing. Briefly spin the tube to collect any drops from the inside wall and tube lid. For PCR products less than 200 bp, add 2 volumes of Buffer GC.
2. Prepare the vacuum manifold according to manufacturer's instructions. Attach the spin column to the manifold**.**
3. Load the **Gel or PCR reaction DNA/Buffer GC solution** to a spin column that is attached to the manifold**.**
4. Turn on the vacuum to let the solution pass through the column.
5. Wash the column by adding **500 µl DNA Wash Buffer.** Repeat once.
6. Place the column in a collection tube and centrifuge at 13, 000 x g for 2 min. It is critical to remove the residual ethanol for optimal DNA yield.
7. Place the column in a clean 1.5 ml tube and add **35-50 µl Elution Buffer** to the column. Incubate at RT for 1 min. Centrifuge the tube at 13,000 x g to elute DNA. Re-apply the eluate to the column for another elution. This increases DNA yield and concentration.

XI. Related Products:

Product Name	Cat. No.
Agarose gel DNA extraction kit	K1441
PCR DNA extraction kit	K1444
Plasmid DNA Purification Kits	K529, K1312 - K1317, K1319 - K1325
Endotoxin Free Plasmid DNA Kits	K1326 - K1336
Mammalian Cell Genomic DNA Isolation Kit	K967
DNA Quantification Assay Kit (Fluorometric)	K539

XII. General Troubleshooting Guide:

Problems	Possible reasons	Suggestions
Low DNA yield	1. Buffer GC was not enough	1. Determine the volume of Buffer GC to be used correctly as instructed. For PCR products less than 200 bp, add 2 volumes of Buffer GC.
	2. Agarose gel did not melt completely	2. Make sure to set the water bath to 55-60°C to allow gel to melt completely. Add more Buffer GC if necessary.
No DNA yield	Forgot to add ethanol to DNA Wash Buffer	Add absolute ethanol to DNA Wash Buffer as instructed before use.
DNA sample floats out of well while loading agarose gel	Ethanol was not completely removed from the column following wash step	After the wash step, centrifuge the empty column with the lid open at top speed for 1 min. Repeat once.

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