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Yeast Genomic DNA Kit

(Cat# K1414-50, -250; Spin Column Based; Store at Multiple Temperatures)

I. Introduction:

BioVision's Yeast Genomic DNA Kit allows rapid and reliable isolation of high-quality total cellular DNA from a wide variety of yeast species. Up to 3 mL of log-phase culture (OD_{600} of 1.0 in YPD medium) can be processed. The system combines the reversible nucleic acid-binding properties of ezBind matrix with the speed and versatility of spin column technology to yield approximately 15-30 µg of DNA with an A_{260}/A_{280} ratio of 1.7-1.9. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques. There are no organic extractions thus reducing plastic waste and hands-on time to allow multiple samples to be processed in parallel. **Yeast Genomic DNA Kit will isolate all cellular DNA, including plasmid DNA.**

II. Sample Types:

- Wide variety of yeast species

III. Kit Contents:

	K1414-50	K1414-250	
Components	**50 Preps**	**250 Preps**	**Part Number**
DNA Mini Columns	50	250	K1414-XX-1
Buffer YTL	20 mL	80 mL	K1414-XX-2
Buffer YBL*	20 mL	80 mL	K1414-XX-3
Buffer KB	28 mL	110 mL	K1414-XX-4
DNA Wash Buffer*	10 mL	3 x 10 mL	K1414-XX-5
Glass Beads	50	250	K1414-XX-6
Elution Buffer	5 mL	50 mL	K1414-XX-7
Buffer SE	30 mL	120 mL	K1414-XX-8
Lyticase**	150 U	750 U	K1414-XX-9
Proteinase K	1 mL	5 x 1 mL	K1414-XX-10
RNase A	120 µL	1.1 mL	K1414-XX-11

**Buffer YBL contains a chaotropic salt. **Add 48 mL (K1413-50) or 200 mL (K1413-250) to each DNA Wash Buffer bottle before use. **Add 2.2 mL SE Buffer to the vial Lyticase for (K1414-50) or 8 mL (K1414-250).*

IV. User Supplied Reagents and Equipment:

- Microcentrifuge capable of at least 13,000g
- Nuclease-free 1.5 mL microfuge tubes
- Water bath set to 30°C
- Shaking water bath set to 55°C; Incubator or water bath set to 65°C
- Absolute (96%-100%) ethanol. ***Do not use other alcohols***

IV. Shipment and Storage:

All components of the Yeast Genomic DNA Kit, except the Proteinase K, RNase A and Lyticase can be stored at 22°C-25°C. Once reconstituted in water, Proteinase K and Lyticase must be stored at -20°C. Store RNase A at 4°C. Under cool ambient conditions, a precipitate may form in the Buffer YBL/YTL. In case of such an event, heat the bottle at 37°C to dissolve. Store Buffer YTL/YBL at room temperature. All Yeast Genomic DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at mentioned above. DO NOT FREEZE!

V. Reagent Preparation and Storage Conditions:

- Add 48 mL (K1413-50) or 200 mL (K1413-250) to each DNA Wash Buffer bottle before use.
- Add 2.2 mL SE Buffer to the vial Lyticase for (K1414-50) or 8 mL (K1414-250)

VI. Yeast Genomic DNA Protocol:

If you are using the Yeast DNA Kit for the first time, please read this manual before beginning the procedure. Yeast cells are grown to log-phase and speroblasts are subsequently prepared. Following lysis, binding conditions are adjusted and the sample applied to a DNA Mini column. Two rapid wash steps remove trace salt and protein contaminants and finally DNA is eluted in water or low ionic strength buffer. Purified DNA can be directly used in downstream applications without the need for further purification.

This method allows genomic DNA isolation from up to 3 mL yeast culture (<$2x10^7$cells).

1. Grow yeast culture in YPD medium to an OD_{600} of 1.0. Harvest no more than 3 mL culture (< $2x10^7$) by centrifugation at 4,000g for 10 min at room temperature.
2. Discard medium and resuspend cells in 480 µL Buffer SE and 40 µL lyticase solution. Incubate at 30°C for at least 30 min.
3. Pellet spheroblasts by centrifuging 10 min at 500g at room temperature.
4. Resuspend cells in 200 µL Buffer YTL. Add Glass beads and vortex for 5 min.
5. Add 20 µL Proteinase K solution and vortex to mix well. Incubate at 55°C in a shaking water bath to complete lysis. Usually no more than 1 h is required for cell lysis. If no shaking water bath is available, incubate and shake or briefly vortex the samples every 20-30 min.
6. Add 5 µL RNase A to samples and invert tube several times to mix. Incubate at room temperature for 5 min.
7. Centrifuge at 10,000g for 5 min to pellet insoluble debris. Carefully aspirate the supernatant and transfer to a sterile micro-centrifuge tube leaving behind any insoluble pellet.
8. Add 220 µL Buffer YBL and vortex to mix at maxi speed for 15 sec. Incubate at 65°C for 10 min. A wispy precipitate may form upon addition of Buffer YBL; it does not interfere with DNA recovery.
9. Add 220 µL absolute ethanol (At room temperature, 96-100%) and mix thoroughly by vortexing at maxi speed for 20 sec. If any precipitation can be seen at this point, break the precipitation by pipetting up and down 10 times.
10. Transfer the entire sample from Step 9 into a DNA column, including any precipitate that may have formed. Centrifuge at 10,000g for 1 min to bind DNA. Discard the the flow-through and reuse the collection tube.
11. Add 500 µL Buffer KB. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.

12. Add 500 µL DNA Wash Buffer. Centrifuge at 10,000 x g for 1 min. Discard flow-through and reuse the collection tube.
13. Centrifuge DNA Mini Column, with the lid open, at maxi speed (⩾10,000g) for 2 min to dry the column. This step is critical for removal of trace ethanol that might otherwise interfere with downstream applications.
14. Place the column into a nuclease-free 1.5 ml microfuge tube and add 50-100 µL of preheated (65 oC) Elution Buffer to DNA Mini column matrix. Allow columns to incubate for 3 to 5 min at room temperature after addition of Elution Buffer.
15. To elute DNA from the column, centrifuge at 10,000g for 1 min.
16. Add the eluted DNA back to the column for a 2nd elution. *NOTE: The first elution typically yields 60-70% of the DNA while the 2nd elution yields another 20-30% of the DNA bound to the column.*

Yeast gDNA isolation vacuum and spin protocol

Note: Please read through previous section of this manual before using this protocol.

1. Prepare samples and column by following the standard Protocol in previous section (Steps 1-11).
2. Prepare the vacuum manifold according to manufacturer's instructions and connect the V-Spin column to the manifold.
3. Load the sample/YBL/Ethanol mixture to the column. Switch on vacuum source to draw the sample through the column and turn off the vacuum.
4. Wash the column by adding 500 µL Buffer KB, draw the Buffer KB through the column by turning on the vacuum source.
5. Wash the column by adding 500 µL DNA Wash Buffer, draw the wash buffer through the column by turning on the vacuum source.
6. Proceed to step 13-16 of Yeast DNA Spin Protocol.

VII. Determination of Yield and Quality:

The total DNA yield can be determined by a spectrophotometer. DNA concentration is calculated as:

[DNA] = (Absorbance260) x (0.05 µg /µL) x (Dilution factor)

The quality of DNA can be assessed by measuring absorbance at both 260 nm and at 280 nm. A ratio of (A260/A280) of 1.7-1.9 corresponds to 85%-95% purity.

VIII. Related Products:

Product Name	Cat. No.	Size
Bacterial Genomic DNA Isolation Kit	K309-100	100 Isolations
Bacterial Genomic DNA Mini Kit	K1388-100, -300	100 Preps, 300 Preps
Bacterial Genomic DNA Mini Kit (w/Lysozyme)	K1389-100, -300	100 Preps, 300 Preps
Concert™ DNA Extraction Kit	K1390-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Genomic DNA Micro Kit	K1385-100, -300	100 Preps, 300 Preps
Genomic DNA Isolation Kit (Cells/Tissues)	K281-50	50 Assays
Genomic DNA Mini Kit (Blood/Cells)	K1380-100, -300	100 Preps, 300 Preps
Genomic DNA Maxi Kit (Blood/Cells)	K1381-10, -25	10 Preps, 25 Preps
Macro Blood DNA Kit	K1392-3, -1000	100 ml, 1000 ml
Macro Blood DNA Plus Kit	K1393-1000	1000 ml
Macro Bacterial DNA Kit	K1394-3, -1000	100 ml, 1000 ml
Macro Bacterial DNA Plus Kit	K1395-1000	1000 ml
Macro Tissue DNA Kit	K1396-3, -1000	100 ml, 1000 ml
Macro Tissue DNA Plus Kit	K1397-1000	1000 ml
Macro Cell DNA Kit	K1398-3, -1000	100 ml, 1000 ml
Macro Cell DNA Plus Kit	K1399-1000	1000 ml
Mammalian Cell Genomic DNA Isolation Kit	K967-100	100 Isolations
Mitochondrial DNA Isolation Kit	K280-50	50 Assays
Plant Genomic DNA Mini Kit	K1383-100	100 Preps
Plant Genomic DNA Maxi Kit	K1384-10, -25	10 Preps, 25 Preps
Plant Tissue Genomic DNA Isolation Kit	K316-100	100 Isolations
Swab Genomic DNA Kit	K1386-100, -300	100 Preps, 300 Preps
Tissue Genomic DNA Mini Kit	K1382-50, -100, -300	50 Preps, 100 Preps, 300 Preps
Triadzol™ Plant DNA Reagent	M1391-100, -200	100 ml, 200 ml
Yeast Genomic DNA Mini Kit	K1387-100, -300	100 Preps, 300 Preps
96 Well Blood Genomic DNA Kit	K1406-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Kit	K1407-2, -4	2 Plates, 4 Plates
96 Well Plant Genomic DNA Binding Plate	M1209-5	5 Plates
Whole Blood DNA Isolation Kit	K528-100	100 Isolations

IX. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Clogged Column	• Incomplete lysis • Sample too large • Incomplete homogenization	• Increase incubation time with Buffer ITL/Proteinase K. An overnight incubation may be necessary. Do not use greater than recommended amount of starting material. For larger samples, divide into multiple tubes. • Pulverize material as indicated in liquid nitrogen to obtain a fine powder.
Low DNA yield or no DNA eluted	• Clogged column • Poor elution • Poor binding to column • Improper washing	• As above. • Repeat elution or increase elution volume. Incubate the column at 70ºC for 5 min before spin • Follow protocol closely when adjusting binding conditions. • DNA Wash Buffer Concentrate must be diluted with ethanol before use.
Low A_{260}/A_{280} ratio	• Extended centrifugation during elution step • Poor cell lysis • Trace protein contaminants remain	• Resin from the column may be present in 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. • Increase incubation time with Buffer ITL. An overnight incubation may be necessary. • Following step 8, wash column with a mixture of [300 µL Buffer BL + 300 µL ethanol] before proceeding to step 9.
No DNA eluted	• Poor cell lysis; Incomplete homogenization • Absolute ethanol not added before adding sample to column • No ethanol added to DNA Wash Buffer Concentrate	• Increase incubation time with Buffer ITL. An overnight incubation may be necessary. • Pulverize starting material as indicated in liquid nitrogen to obtain a fine powder. • Before applying DNA sample to column, add Buffer BL and absolute ethanol. • Dilute Wash Buffer with the indicated volume of absolute ethanol before first use.

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