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

Fungal Genomic DNA Kit

(Cat# K1415-50, -250; Store at RT)

I. Introduction:

BioVision's Fungal Genomic DNA Kit is designed for efficient recovery of genomic DNA up to 60 kb in size from fresh and dried Fungal tissue samples. Up to 100 mg of wet tissue (or 30 mg dry tissue) can be processed in less than 1 hour. The system combines the reversible nucleic acid-binding properties of the matrix with the speed and versatility of spin column technology to eliminate polysaccharides, phenolic compounds, and enzyme inhibitors from Fungal tissue lysates. Purified DNA is suitable for PCR, restriction digestion, and hybridization techniques.

Samples are homogenized and lysed in a high salt buffer. The DNA is bound to the column while proteins and other impurities are removed by wash buffer. The purified DNA is suitable for downstream applications such as endonuclease digestion, thermal cycle amplification, and hybridization techniques.

II. Sample Types:

- Wide variety of Fungal species and tissues

III. Kit Contents:

	K1415-50	K1415-250	
Components	**50 Preps**	**250 Preps**	**Part Number**
DNA Columns	50	250	K1415-XX-1
Buffer FP1	40 mL	180 mL	K1415-XX-2
Buffer CFP1	30 mL	110 mL	K1415-XX-3
Buffer FP2	12 mL	50 mL	K1415-XX-4
Buffer FP3	15 mL	200 mL	K1415-XX-5
RNase A	100 µL	500 µL	K1415-XX-6
DNA Wash Buffer*	12 mL	50 mL	K1415-XX-7
Elution Buffer	15 mL	55 mL	K1415-XX-8

Add 48 mL (K1415-50) or 200 mL (K1415-250) to each DNA Wash Buffer bottle before use.

IV. Shipment and Storage:

All components of the Fungal Genomic DNA Kit, except the RNase A can be stored at 22°C-25°C. Store RNase A at 4°C. During shipment, or storage in cool ambient conditions, precipitates may form in Buffer FP1 and Buffer FP2. It is possible to dissolve such deposits by warming the solution at 37°C, though it doesn't interfere with the overall performance. DO NOT FREEZE!

V. Reagent Preparation and Storage Conditions:

- Dilute Wash Buffer Concentrate with ethanol and store at room temperature. Add 48 mL (K1415-50) or 200 mL (K1415-250) absolute (96%-100%) ethanol to each bottle.
- Choose the most appropriate protocol to follow. Procedures are described for dried and fresh (or frozen) specimens.
  A. Dry Specimens for processing ≦ 50 mg powdered tissue. DNA yields range from 50 µg-100 µg per 100 mg dry tissue.
  B. Fresh or Frozen for processing ≦ 200 mg fresh (or frozen) powdered tissue. DNA yields range from 50 µg to 100 µg per 100 mg dry tissue.

VI. Fungal Genomic DNA Protocol:

Dry Specimens Protocol:

Materials supplied by user:

- Microcentrifuge capable of at least 12,000 rpm.
- Nuclease-free 1.5 mL and 2.0 mL centrifuge tubes.
- Waterbath equilibrated to 65 °C.
- Equilibrate sterile water or Elution Buffer at 65 °C.
- Absolute (96%-100%) ethanol.
- Optional: 2-mercaptoethanol.

This is the most robust method for isolation of total cellular (mitochondrial, chloroplast, and genomic) DNA. Yields are usually sufficient for several tracks on a Southern blot for RFLP mapping. Drying allows storage of field specimens for prolonged periods of time prior to processing. Samples can be dried overnight in a 45 °C oven, powdered, and stored dry at room temperature. To prepare dried samples, place ≦50 mg of dried tissue into a 2.0 mL microfuge tube and grind using a pellet pestle. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until clean. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and wiping the surface clean between samples. A fine powder will ensure optimal DNA extraction and yield. Process four to six tubes as a group till Step 2 and start another set.

1. To 10-50 mg powdered dry tissue, add 600 µL Buffer FP1 in a 2.0 mL microfuge tube. Optional: Add 10 µL ß-mercaptoethanol and vortex vigorously to mix. Make sure to disperse all clumps. *Note: Process in sets of four to six tubes: grind, add Buffer FP1 and ß-mercaptoethanol, and proceed to Step 2 before starting another set. Initially, do not exceed 50 mg dried tissue. Amount can be increased according to results.*
2. Incubate at 65 °C for 10 min. Mix sample twice during incubation by inverting tube. *Optional: If necessary, add 2 µL of RNase into the lysate before incubation to remove the RNA.*
3. Add 200 µL Buffer FP2 and mix well by vortexing for 10 sec. Incubate on ice for 5 min. Centrifuge at 13,000 rpm for 5 min.
4. Carefully transfer the supernatant to a clean 2.0 mL tube. Add 0.7 volume of isopropanol. Mix well by vortexing for 5s and centrifuge at 12,000 x g for 2 min to precipitate the DNA. *Note: This step removes polysaccharides and improves DNA binding ability to the spin column.*

5. Carefully decant the supernatant and discard, avoid dislodging the DNA pellet. Inverting the tube on absorbance paper towel for 1 min to drain any residual ethanol. The pellet doesn't have to be dry.
6. Add 300 µL preheated (65°C) ddH_2O and vortex for 10s to mix the DNA well. *Note: A brief incubation at 65°C may help dissolve the DNA.*
7. Add 150 µL of Buffer FP3 and 300 µL 100% ethanol, mix well by vortexing for 5s. A precipitation may form but it does not interfere with the DNA binding to the column.
8. Transfer the sample to a column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and put the column back in the collection tube.
9. Add 500 µL DNA Wash Buffer and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube. Repeat once and put the column, with the lid open, back to the collection tube.
10. Centrifuge the column at 13,000 rpm for 1 min. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
11. Transfer column to a clean 1.5 mL tube. Add 100 µL Elution Buffer (or sterile deionized water) pre-warmed to 65 ℃ and centrifuge at 13,000 rpm for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields. Use of more than 200 µL of buffer for elution is not recommended.
12. Optional: Add the eluted DNA back to the column for another elution. The first elution normally yields 60-70% of the DNA while a second elution yields 20-30% of the DNA.

Fresh or Frozen Specimens Protocol:

Materials supplied by user:

- Microcentrifuge capable of 12,000 rpm
- Nuclease-free 1.5 mL and 2.0 mL microfuge tubes
- Water bath equilibrated to 65℃
- Equilibrate sterile water or Elution Buffer at 65℃.
- Absolute (96%-100%) ethanol
- Liquid nitrogen for freezing/disrupting samples
- Optional: 2-mercaptoethanol

NOTE: Use extreme caution when handling liquid nitrogen.

This protocol is suitable for most fresh or frozen tissue samples allowing more efficient recovery of DNA. However, due to the tremendous variation in water and polysaccharide content of Fungals, sample size should be limited to≦200 mg. Best results are obtained with young leaves or needles. To prepare samples, collect tissue in a 1.5 mL or 2 mL microfuge tube and freeze by dipping in liquid nitrogen with a pair of tweezers to fill the tube. Grind the tissue using disposable kontes pellet pestles, which are available from VWR. Alternatively, one can allow liquid nitrogen to evaporate and then store samples at -70℃ for later use. For critical work such as PCR and cloning, pestles are best used a single time then soaked in a dilute bleach solution immediately after use until cleaning. Disposable pestles may be autoclaved several times. For standard Southern analysis, the same pestle can be reused several times to grind multiple tissue samples by rinsing with ethanol and carefully wiping the surfaces clean between samples.

1. Collect ground fungal tissue as described (start with 100 mg) in a 2.0 mL microfuge tube and immediately add 600 µL Buffer FP1. Optional: Add 10 µL ß-mercaptoethanol and vortex vigorously. Make sure to disperse all clumps. DNA cannot be effectively extracted from clumped tissue. TIP: Process in sets of four to six tubes: fill all tubes with liquid nitrogen, grind, and add Buffer FP1 and 2-mercaptoethanol; proceed to Step 2 before starting another set. As a starting point, use 100 mg tissue per tube and if yield and purity are satisfactory increase to 200 mg.
2. Incubate at 65℃ for 10 min. Mix sample twice during incubation by inverting tube. Optional: If necessary, add 2 µL of RNase into the lysate before incubation to remove the RNA.
3. Add 140 µL Buffer FP2 and mix well by vortexing for 10s. Centrifuge at 13,000 rpm for 10 min.
4. Carefully transfer the supernatant to a clean 2.0 mL tube. Add 0.7 volume of isopropanol. Mix well by vortexing for 5s and centrifuge at 12,000 x g for 2 min to precipitate the DNA.
   Note: This step removes polysaccharides and improves DNA binding ability to the spin column.
5. Carefully decant the supernatant and discard, avoid dislodging the DNA pellet. Inverting the tube on absorbance paper towel for 1 min to drain any residual ethanol. The pellet doesn't have to be dry.
6. Add 300 µL preheated (65°C) Elution Buffer and vortex for 10s to mix the DNA well. NOTE: A brief incubation at 65°C may help dissolve the DNA.
7. Add 150 µL of Buffer FP3 and 300 µL 100% ethanol, mix well by vortexing for 5 sec. A precipitation may form but it does not interfere with DNA binding to the column.
8. Transfer the sample to a DNA column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through and put the column back to the collection tube.
9. Add 500 µL DNA Wash Buffer and centrifuge at 13,000 rpm for 30 sec. Discard the flow-through and put the column back to the collection tube. Repeat once. Put the column, with the lid open, back to the collection tube.
10. Centrifuge the column at 13,000 rpm for 1 min. This step is critical for removing residual ethanol that may otherwise be eluted with DNA and interfere with downstream applications.
11. Transfer the column to a clean 1.5 mL tube. Add 100 µL Elution Buffer pre-warmed to 65 ℃ and immediately centrifuge at 13,000 rpm for 1 min to elute DNA. Smaller volumes will significantly increase DNA concentration but give lower yields. The first elution normally yields 70% of the DNA bound to the column.
12. Optional: Add the eluted DNA back to the column for a 2nd elution yields another 20-30% of the DNA bound. ***Note****: To increase DNA concentration, add Elution Buffer and incubate the column at 60℃-70℃ for 5 min before elution.*

Protocol for DNA isolation from Samples rich in polyphenols and/or polysaccharides:

1. Collect ground Fungal tissue (start with 100 mg) in a 2.0 mL microfuge tube and immediately add 500 µL Buffer CFP1.

2. Incubate at 65℃ for 15 min. Mix sample twice during incubation by inverting tube. Optional: If necessary, add 2 µL of RNase into the lysate before incubation to remove the RNA.
3. Add 800 µL chloroform /Isoamyl alcohol (24:1) and vortex to mix. Centrifuge at ≧10,000 x g for 5 min. Transfer 300 µL of supernatant to a new tube making sure not to disturb the pellet or any precipitates.
4. Add 150 µL of Buffer FP3 followed by 300 µL of absolute ethanol and vortex to obtain a homogenous mixture. A precipitate may form upon addition of ethanol; it will not interfere with DNA isolation.
5. Apply the entire sample to a DNA column with the collection tube. Centrifuge at 13,000 rpm for 1 min to bind DNA. Discard the flow-through liquid and reuse the collection tube.
6. Add 500 µL of DNA Wash Buffer to the column and centrifuge at 13,000 rpm for 1 min. Discard the flow-through liquid and reuse the collection tube. Repeat once.
7. Centrifuge the column at 13,000 rpm (top speed) for 1 min.
8. Transfer the column to a clean 1.5 mL tube and add 100 µL of Elution Buffer (Pre-warmed to 65℃) and incubate at room temperature for 1 min.
9. Centrifuge at 13,000 rpm for 1 min to elute the DNA. Optional: Add the eluted DNA back to the column for another elution. The first elution normally yields 60-70% of the DNA while a second elution yields 20-30% of the DNA.

VII. 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

VIII. General Troubleshooting Guide:

Problems	Possible Reasons	Solutions
Clogged well	• Carry-over of debris. • DNA pellet not completely dissolved before applying sample to column. • Sample too viscous. • Incomplete precipitation following addition of FG 2.	• Following precipitation with Buffer FG 2, make sure no particulate material is transferred. • In protocols A and B, ensure that DNA is dissolved in water before adding Buffer FG 3 and ethanol. This may need repeated incubation at 65℃ and vortexing. • In protocol C, do not exceed suggested amount of starting material. Alternatively, increase amounts of Buffers FG 1 and FG 2 and use two or more columns per sample. • Increase RCF or time of centrifugation after addition of Buffer FG 2.
Low DNA yield or no DNA eluted	• Incomplete disruption of starting material. • Poor lysis of tissue. • DNA remains bound to column. • DNA washed off.	• For both dry and fresh samples, obtain a fine homogeneous powder before adding Buffer FG 1. • Decrease amount of starting material or increase amount of Buffers FG 1 and FG 2. • Increase elution volume to 200 µL and incubate on column at 65℃ for 5 min before centrifugation. • Dilute Wash Buffer Concentrate by adding appropriate volume of absolute ethanol prior to use (page 3).
Problems in downstream applications	• Salt carry-over • Ethanol carry-over	• DNA Wash Buffer must be at room temperature. • Following the second wash spin, ensure that the column is dried by centrifuging 2 min at maximum speed.

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