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Mycoplasma Genomic DNA Kit

(Cat# K1416-50, -250; Store at RT)

I. Introduction:

BioVision's Mycoplasma Genomic DNA Kit provides a fast and easy method for isolating Genomic DNA from Mycoplasma. The system utilizes the reversible nucleic acid-binding properties of ezBind membrane and the speed of spin column technology to yield high quality Genomic DNA with the $OD_{260/280}$ ratio of 1.7-1.9. Purified DNA is ready for applications such as PCR, Southern Blotting, and Restriction Digestion.

II. Sample Types:

- Mycoplasma

III. Kit Contents:

	K1416-50	K1416-250	
Components	**50 Preps**	**250 Preps**	**Part Number**
DNA Mini Columns	50	250	K1416-XX-1
Buffer TLY	15 mL	75 mL	K1416-XX-2
Buffer KB	28 mL	140 mL	K1416-XX-3
DNA Wash Buffer*	15 mL	50 mL	K1416-XX-4
Elution Buffer	15 mL	60 mL	K1416-XX-5
Protease K	1 mL	5 x 500 µL	K1416-XX-6
RNase A	240 µL	1.4 mL	K1416-XX-7

Add 45.0 mL (K1416-50) or 200 mL (K1416-250) to each DNA Wash Buffer bottle before use.

IV. User Supplied Reagents and Equipment:

- Tabletop Microcentrifuge
- Nuclease-free 1.5 mL microfuge tubes
- Water bath; Equilibrate sterile ddH_2O water at 65℃
- Absolute (96%-100%) ethanol. Do not use other alcohols
- PBS Buffer

V. Shipment and Storage:

All components of the Mycoplasma Genomic DNA Kit, except the RNase A can be stored at 22°C-25°C. Store RNase A at 4°C. All Mycoplasma Genomic DNA Kit components are guaranteed for at least 12 months from the date of purchase when stored at 22°C - 25°C. DO NOT FREEZE!

VI. Reagent Preparation and Storage Conditions:

- Aliquot and store vials of reconstituted Protease K at - 20°C.
- Dilute DNA Wash Buffer with absolute ethanol as follows: Add 45 mL (K1416-50) or 200 mL (K1416-250) of absolute ethanol to each bottle. The final concentration is 70%.
- Under cool ambient conditions, precipitates may form in Buffer LY. In case of such an event, heat the bottle at 37°C to dissolve before use.

VII. Mycoplasma Genomic DNA Protocol:

All centrifugation steps must be carried out at room temperature

1. Transfer the sample to a 1.5 mL tube and bring the volume up to 250 µL with PBS or Elution Buffer (provided) if the sample volume is less than 250 µL.
2. Add 20 µL of protease K and 250 µL of Buffer LY. Mix well by vortexing at maximum speed for 10 sec. If RNA-Free genomic DNA is required, add 5 µL of RNase A (provided) to each sample. ***Note: Precipitates may form in Buffer LY. Dissolve at 37°C before use.***
3. Incubate the sample at 65°C for 10 min. Briefly vortex the tube once during incubation.
4. Add 250 µL of absolute ethanol to the lysate. Vortex at maximum speed for 10 sec. Briefly centrifuge the tube to collect any drops from the lid.
5. Insert a DNA column into a collection tube. Transfer the sample to the DNA column, and centrifuge at 12,000 rpm for 30 s. Discard flow-through liquid.
6. Place the column back into the collection tube. Add 500 µL of Buffer KB, and centrifuge at 12,000 rpm for 30 s. Discard flow through liquid and put the column back to the collection tube.
7. Add 600 µL of DNA Wash Buffer (Add ethanol before use). Centrifuge at 12,000 rpm for 30s. Discard flow-through liquid. ***Note: DNA Wash Buffer is provided as a concentrate and must be diluted with absolute ethanol.***
8. Put the empty column, with the lid open, into the same 2 mL collection tube and centrifuge at 12,000 rpm for 1 min to dry the column. *Note:Residual ethanol will be removed more efficiently with the lid of the column open. It is critical to remove the ethanol before elution.*
9. Place the column into a sterile 1.5 mL microtube, add 100 µL (preheated at 65°C) Elution Buffer. Incubate at room temperature for 2 min.
10. Centrifuge at 13,000 rpm for 1 min to elute the DNA. The first elution normally yields 60-70% of DNA bound.
11. Optional: Elute the column with another 100 µL (preheated at 65°C) Elution Buffer. The second elution will yield another 20% of the DNA bound.

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