Quick Bacteria Genomic DNA Extraction Kit

Cat. No: KBN101152
Size: 100 preps
Store at: -20°C

For research use only

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Quick Bacteria Genomic DNA Extraction Kit

Description:

The quick bacteria genomic DNA extraction system uses the silica-based membrane technology for simple and fast isolation of genomic DNA (gDNA) without phenol/chloroform. Homogenization is not necessary since tissues are directly lysed by Proteinase K. The buffer system is optimized to allow selective binding of DNA to the silica-based membrane. The simple centrifugation procedure can completely removes impurities such as proteins, divalent cations, and secondary metabolites. Pure DNA is then eluted in water or low-salt buffer, ready to use.
Genomic DNA Purification Kit is suitable for high quality genomic DNA purification of tissues from Gram Positive and Gram Negative Bacteria.

Materials Provided:

Solution DS	30 ml
Solution MS	40 ml
Proteinase K (20mg/ml)	2 ml
Wash Buffer PS	60 ml
Wash Buffer PE	30 ml
Elution Buffer TE (10mM Tris-HCl, 1mM EDTA, pH 8.5)	10 ml
Spin Columns	100 each

Downstream Applications:

Purified DNA is free of impurities and enzyme inhibitors, and have an $_{A260/280}$=1.7-1.9, is suitable for applications such as:

- Genotyping
- PCR/qPCR
- Restriction enzyme digestion
- Sequencing
- Southern blotting

Features:

- **Fast** - procedure takes only 60 min
- **High efficiency** - 3-20 ug of genomic DNA from 1-5 – 2 ml bacteria culture
- **Safe** - no phenol/cholroform extraction step
- **High purity** - purified DNA without enzyme inhibitors, RNA or proteins, ready for downstream applications

Storage:

Store Proteinase K at -20°C, other reagents at room temperature for up to 1 year.
Any precipitate in the Solution DS and Solution MS can be dissolved by incubating at 37°C before use.

Important Notes:

- Prior to the initial use of the kit, dilute the Wash Buffer PE with ethanol (96-100%):

Solution	**KBN101162 (100 preps)**
Wash Buffer PE	30 ml
Ethanol	90 ml
Total Volume	120 ml

Mix well, mark the labels on the bottle that ethanol is added.

- Ensure that no DNases are introduced into the sterile solutions of the kit.
- Make sure there is no precipitates in Solution DS and Solution MS. If any precipitate is visible, warming the solutions at 37°C for 3-5 min to dissolve the precipitate, and cooling to 25°C before use.
- Wear disposable gloves when handling the Solution MS as it contains guanidine hydrochloride.
- All purification steps should be carried out at room temperature.
- All centrifugations should be carried out by a table-top microcentrifuge at >12,000 g (10,000-14,000 rpm, depending on the rotor type).

Protocol:

1. Add 0.5 – 2 ml of overnight bacteria culture (~2 × 10^9 cells) to a 1.5 ml microcentrifuge tube. Centrifuge at 12,000 rpm for 1 min to pellet the cells. Remove the supernatant.

2. Add 200 ul **Solution DS**. Mix immediately and thoroughly by brief vortexing or inverting.
   - For Gram Positive Bacteria, add 108 ul Solution DS and 72 ul lysyme (50 mg/ml), mix well by brief vortexing or inverting. Incubate at 37℃ for 30 min.

   Optional • If RNA-free genomic DNA is required, add 4 ul RNase A (100 mg/ml) and incubate for 5 min at room temperature. RNase A can be purchased separately.

3. Add 20 ul **Proteinase K**, Mix thoroughly by brief vortexing or inverting. Incubate at 55°C until yield a homogeneous solution (~30 min).

4. Add 220 ul **Solution MS**, Mix thoroughly by brief vortexing or inverting. Incubate at 65°C for 10 min (inverting several times to yield a homogeneous solution).

5. Add 220 ul **ethanol (96–100%)** to the lysate, and mix thoroughly by brief vortexing or inverting.

6. Pipet the mixture from step 5 into the spin column placed in a 2 ml collection tube (provided). Centrifuge at 12,000 rpm for 1 min. Discard flow-through.
   - Genomic DNA is adsorbed on the silica membrane of the column in this step.

7. Add 500 ul **Wash Buffer PS**, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.

8. Add 500 ul **Wash Buffer PE**, and centrifuge for 1 min at 12,000 rpm. Discard flow-through.
   - Wash Buffer PE must be diluted with ethanol (96-100%) previously.

9. Repeat step 8.

10. Centrifuge for 3 min at 12,000 rpm to dry the column membrane. Discard flow-through and collection tube.
    - Since residual ethanol may interfere with subsequent reactions, it is important to dry the membrane of the spin column. This centrifugation step ensures that no residual ethanol will be carried during the following elution step. If carryover of ethanol occurs, empty the collection tube, then reuse it after centrifuging for 1 min at 12,000 rpm.

11. Place the spin column in a clean 1.5 ml microcentrifuge tube (not provided), and pipet 30-100 ul **Elution Buffer TE** directly onto the membrane. Incubate at room temperature for 2 min.
    - Elution buffer TE can be replaced by deionized water. But the pH should be 8.0-8.5.
    - Prewarm Elution Buffer TE to 65°C can increase the yield of genomic DNA.

12. Centrifuge for 2 min at 12,000 rpm. The tube contains the purified DNA. **Store the DNA at -20°C.**

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