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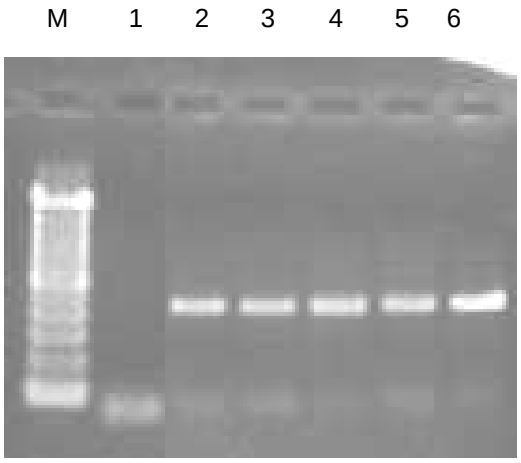
User's Manual and Instructions

Bacteria Identification Kit

Catalog Number: K5081100

Introduction
Bacteria identification kit is an ultra-sensitive and universal system developed by Biochain to identify bacteria by Polymerase Chain Reaction (PCR). It detects bacteria at concentration of 0.2 or more cfu/per PCR reaction.



Agarose gel electrophoresis of PCR products from using 10 bacteria cell lysate as template.
Lane 1: Negative control, lysis buffer only
Lane 2: Escherichia Coli
Lane 3: Bacillus Cereus
Lane 4: Klebsiella Pneumoniae
Lane 5: Streptococcus Agalactiae
Lane 6: Staphylococcus Aureus
Size 475 bp PCR product was detected by a pair of 16S specific PCR primers.

Features
- **Sensitive -** detects bacteria at concentration of 0.2 or more cfu/per PCR reaction.
- **Simple** - bacteria is directly lysed and applied in PCR
- **Convenient** - Complete kit contains universal lysis buffer, PCR mix, and universal control primers of 16S gene.
- **Versatile** - Detect different types of Gram negative or positive rod and coccus bacteria
- **Cost-effective –** No extraction and purification of Bacteria DNA is needed
- **Compatible** - can be incorporated into other PCR and detection systems such as Real Time PCR, fluorescence detection, multiplex PCR, nest PCR system

Applications
- Detects and identifies trace amount of bacteria
- Identify recombinant gene either on genome or plasmid inside bacteria
- Incorporated into other PCR and detection systems such as Real Time PCR, fluorescence detection, multiplex PCR, nest PCR system and etc.
- Potential in clinical applications

Description
This kit consists of universal lysis buffer, PCR mix, and universal control primers for 100 25 µl volume PCR reactions. This system can detect different types of Gram negative or positive rod and coccus bacteria, such as **Escherichia Coli, Klebsiella Pneumoniae, Bacillus Cereus, Staphylococcus Aureus, Streptococcus Agalactiae,** and etc. With the powerful Universal lysis buffer, the sensitivity

of this system is so high that bacteria DNA can be detected by PCR directly from lysing the bacteria without further DNA extraction and purification. Beside conventional PCR and agarose gel electrophoresis detection, this system can be incorporated into other PCR and detection systems such as Real Time PCR, fluorescence detection, multiplex PCR, and nest PCR system.

Quality Control
Using bacteria lysate from 100, 10 and 0 Escherichia Coli cell as template, PCR amplify the 16s gene with control primers. A 475 bp PCR products are detected in 100 and 10 cell lysate but not in 0 cell lysate.

Kit Components

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Item	Amount	Part#
1. 10x Universal lysis buffer	1 ml	K5081100-1
2. 2x PCR mix (MgCl, dNTP, Taq polymerase, and pH control reagent)	1.25 ml	K5081100-2
3. Universal Control Primers (Mixture of Forward and Reverse Primers from 16S gene, 5 µM each)	100 µl	K5081100-3

Storage and Stability
Dry ice shipping and store at -20°C. The kit is stable for one year when handled properly.

Items not supplied:
1. DNA and bacteria free water or double distilled water.
2. PCR tubes
3. PCR instrument
4. Agarose gel
5. Polyacrylamide
6. DNA staining dye.
7. E.Coli bacteria.

Recommended Protocol:
1. Centrifuge 1 ml of **testing specimen** containing bacteria (1 to 1x 10^6 cfu/ml) in micro centrifuge tube at 11000 RPM for 3 minutes, pour out supernatant as much as possible, and save bacteria pellet. See following table to estimate amount of bacteria
   Option: prepare an extra specimen as **positive control** by using Universal Control Primers later.

Specimen Format	Amount of Specimens
Over night bacterial culture	0.5 µl-2 µl
Bacterial colony on plate	1/10-1/2 of Colony
Unknown bacteria contaminated specimen	1 ml try first
Purified bacteria genomic DNA as positive control	10 ng

2. Add 90 µl DNA and bacteria free water or double distilled water into micro centrifuge tube from Step 1 to re-suspend bacteria pellet.
   Option: add 90 µl DNA and bacteria free water or double distilled water into a blank micro centrifuge tube as **negative control** by using Universal Control Primers later.

3. Add 10 µl of 10x Universal lysis buffer into testing bacteria suspension and control specimens from Step 2.
4. Heat testing bacteria suspension and control specimens from Step 3 at about 100°C in heat block for 10 minutes.
5. Prepare PCR amplification as following table:
   Option: You may use your own PCR reagents, add labeled nucleotide or scale up or scale down reaction volume.

	Testing Specimens	Positive Control	Negative Control
2x PCR mix	12.5 µl	12.5 µl	12.5 µl
Universal control primers		1 µl	1 µl
Your primers (5 µM each)	0.5 µl each		
Testing Specimen	11.5 µl		
Positive control		11.5 µl	
Negative control			11.5 µl
Total volume	25 µl	25 µl	25 µl

6. Suggested PCR cycle:

Temperature	Time (seconds)	Cycle
95°C	120	1
95°C	30	35
56°C	45	
72°C	30	
72°C	600	1
4°C	Option	1

7. Apply 5 µl of PCR products on agarose gel or polyacrylamide to perform gel electrophoresis. Staining agarose gel with solution containing 0.5 µg/ml Ethidium Bromide for about 10 min. Then analyses result under UV light.
8. You will use your own detection system to analyses result if you choose your own PCR protocols.
9. Analysis of results: A 475 bp PCR product should be shown from the positive control. And no PCR product is shown from the negative control.

Trouble Shooting

1. General Problems

1.1 No PCR products in all Samples

- Inhibiting material from specimens to inhibit PCR. **Add less specimens**
- Too much specimens used. **Add less specimens**
- Less bacteria in specimens. **Add more specimens or test E.Coli**
- Use your own PCR cocktail. **Add PCR mix included in this kit.**
- The Kit is not working on your specific bacteria. **Isolate DNA from your bacteria, and use the DNA as template for a PCR reaction**

1.2 Only positive control has PCR products

- Your primers failed. **Check your primers with your own positive control**
- Less bacteria in specimens when test low copy gene. **Add more specimens.**

1.3 Negative control has PCR products

- Contamination of DNA and Bacteria Free water or double distilled water. **Use new water**
- Contamination of reagent provided in the kit **Get a new Kit**.

2. Specific Problems

2.1 Your bacteria is not working

- Your bacteria work with control primers only. **Checker your primers.**
- Your bacteria do not work with control primers. **Isolate DNA from your bacteria, and use the DNA as template for a PCR reaction**

2.2 Your PCR products are not perfect when using PCR mix included in the Kit

- Amount of your own primers is not optimized. **Titration of your primers**
- Your primers are not specific. **Redesign your primers**

2.3 Your PCR products are not perfect when using your own PCR reagent

- You do not have to use your own PCR cocktail. **Try PCR mix included in the Kit.**

Related Products

Express Cloning Checker Kit, K5013200

Appendix:

Preparation of material not supplied with kit:

Material	Preparation	Stability/temperature	Notes
DNA and Bacteria Free water or double distilled water	Double distill water going through 0.22 µm filter and autoclave.	3 months at RT	Do not repeat using the same water aliquot.
Grow E Coli. Bacteria	Growing E. Coli in 2 ml of LB bacteria culture medium over night	Freshly made suggested	Can be Stored at 2-8°C O/N or -70°C for once
Agarose gel	2% agarose gel in 1x TAE, or 1x TBE buffer if your PCR products are less than 500 bp	2 hours at RT and two days at 4°C	Easy to prepare
Polyacrylamide gel	5% Polyacrylamide gel in 1x TBE	2 hours at RT and two days at 4°C	Nice result with sharper bands
DNA Staining Solution	Make Ethidium Bromide at 0.5 µg/ml in 1x TAE or 1x TBE	3 days at RT and 6 months at 4°C in high concentration (10 mg/ml)	Mutagenic material Consult your safety officer.