Microorganism Direct PCR Kit

Quick preparation of template DNA from microorganism for PCR without DNA Isolation

Kit Contents

Cat. No.	TQ3100-01	TQ3100-02	TQ3100-03
Preps	20 preps	100 preps	500 preps
MP1 Buffer	1 ml	6 ml	30 ml
MP2 Buffer	1 m	6 ml	30 ml
2 x Taq Master Mix	1 ml	2 x 1 ml	8 x 1 ml
Distilled water	2 ml	10 ml	50 ml

Shipping and Storage

The Microorganism Direct PCR Kit is shipped at 2-8℃. 2 x Taq Master Mix should be stored at -20℃.

Product Description

The Microorganism Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from all kinds of gram-positive, gram-negative, fungal, actinomycetes samples, like *E.coli, pseudomonas aeruginosa, enterococcus faecalis, staphylococcus aureus* or *rhizopus, aspergillus, saccharomyces cerevisiae, pennicillium* etc. Briefly, the DNA is extracted from a sterilization toothpick which picking colony or hypha from platingmedium, a little microorgamism sample incubation in the Extraction Solution at room temperature, then put it in 90°C for 5 minutes. After an equal volume of the Dilution Solution is added to the extract to neutralize inhibitory substances, the extract is ready for PCR. An aliquot of the diluted extract is then combined with the 2 x Taq Master Mix and user provided PCR primers to amplify target DNA. 2 x Taq Master Mix is a 2 x Reaction Mix containing buffer, salts, dNTPs, and Taq DNA Polymerase. It is optimized specifically for use with the extraction reagents.

Protocol for Microorgamism Extraction

1. **Pipette 50 ul MP1 buffer into a 2 ml collection tube or suitable vessel**, used sterilization toothpick picking the colony or hypha from platingmedium, put it into the MP1 buffer and a few stir gently, discard the toothpick.

   Close the tube and vortex briefly. Make sure the sample is covered by the Extraction Solution.

2. Incubate at 95°C for 5 minutes.

3. **Add 50 µl MP2 Buffer and vortex to mix.**

4. Store the extraction at 2-8°C.

PCR Protocol

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions, such as incubation times, temperatures, and amount of template DNA, may vary and must be individually determined.

1. **Thaw primer solutions.** Keep on ice after complete thawing, and mix well before use.
2. **Mix the Taq Master Mix by vortexing briefly.** It is important to mix the Taq PCR Master Mix before use to avoid localized differences in salt concentration.
3. Prepare one of the following reaction mixes on ice: (For a 25 µl reaction volume)

Component	Volume	Final Concentration
2X Taq Master Mix	12.5 µl	1X
Upstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
Downstream Primer, 10 µM	0.5 µl	0.1–1.0 µM
DNA Template	4 µl	<500 ng
Nuclease-Free Water to		25 µl

4. Gently mix the reaction and spin down in microcentrifuge.
5. **Set up program for a routine PCR reactions:**

Initial Denaturation	94-95°C for 1-5 min
25-40 cycles	94-95°C for 30 sec
	45-70°C for 10-30 sec
	72°C for X min(1min/kb)
Final extension	72°C for 7 min
Final soak	4-10°C

6. For a simplified hot start, proceed as described in step 7. Otherwise, place the PCR tubes in the thermal cycler and start the cycling program.
7. **Simplified hot start:** Start the PCR program. Once the thermal cycler has reached 94°C, place the PCR tubes in the thermal cycler. In many cases, this simplified hot start improves the specificity of the PCR.