Plant Direct PCR Kit

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

Kit Contents

Cat. No.	TQ2800-01	TQ2800-02	TQ2800-03
Preps	20 preps	100 preps	500 preps
PT1 Buffer	2 ml	12 ml	60 ml
Proteinase K	3mg	11mg	5 x 11mg
PT2 Buffer	1 ml	1 ml	5 x 11ml
PT3 Buffer	2 ml	12 ml	60 ml
2 x PCR Master Mix	1 ml	2 x 1 ml	8 x 1 ml
Distilled water	2 ml	10 ml	50 ml

Shipping and Storage

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

Product Description

The Plant Direct PCR Kit contains all of the reagents required to rapidly extract and amplify genomic DNA from plant leaves. Briefly, the DNA is extracted from a piece of leaf tissue, a 0.5 to 0.7cm disk cut with a standard paper punch, by incubation in the Extraction Solution at 55°C for 10 minutes, then put it in 95°C for 5 minutes. After an equal volume of the PT3 Buffer 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 PCR MasterMix and user provided PCR primers to amplify target DNA. 2 x PCR MasterMix 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 Plant Extraction

Prepare Proteinase K Solution: Add PT2 Buffer to dissolve Proteinase K and Store at -20°C.

TQ2800-00: Add 120 µl PT2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2800-01: Add 550 µl PT2 Buffer to the tube of Proteinase K, gently mix to dissolve Proteinase K.

TQ2800-02: Add 550 µl PT2 Buffer to each tube of Proteinase K, gently mix to dissolve Proteinase K.

1. **Rinse the paper punch and forceps in 70% ethanol prior to use** and between the handing of different samples.
2. **Punch a 0.5-0.7 cm disk of leaf tissue into a 2 ml collection tube or suitable vessel** using a standard one hole paper punch. If frozen plant tissue is used, keep the leaves on ice while punching disks.
3. **Add 95 µl PT1 Buffer and 5 µl PT2 buffer to the collection tube.** Close the tube and vortex briefly. Make sure the disk is covered by the Extraction Solution.
4. Incubate at 56°C for 10-30 minutes.
5. Incubate at 95°C for 10 minutes.
6. **Add 100 µl PT3 Buffer and vortex to mix.**
7. 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 PCR Master Mix by vortexing briefly.** It is important to mix the 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 PCR 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.