1-Drop PCR Master Mix (with dye, squeeze bottle design)

Cat. #: W2599-5 (5 mL), W2599S (0.5 mL)

Ship / Storage: Ship at room temperature. Store at -20℃ for up to 1 year and avoid freeze-thaw cycles. Stored at 4℃ **for up to 6 months**.

Product Components

Components	Cat.#: **W2599-5**	Cat.#: **W2599S**
1-Drop PCR Master Mix in squeeze bottle	5 mL	0.5 mL test sample

Application

- Routine PCR
- T/A cloning

Product Description (This product is for research use only.)

Easy handling: squeeze then run PCR.

User can squeeze one drop of PCR Master Mix into PCR tube/plate, add template and primers, and then can run PCR reaction. This PCR Master Mix contains Taq DNA Polymerase, PCR buffer, Mg2+, dNTP, PCR stabilizer and PCR enhancer. This unique Mix recipe makes the system very **reliable, and tolerant to most of the PCR condition** (concentration of the components).

The Taq Polymerase in the PCR Master Mix is **Hot Start** polymerase, which activity is inhibited at ambient temperatures by the chemical modificaiton. This prevents the formation of misprimed products and primer dimers at ambient temperatures. So no need to prepare the reactions on ice. The Polymerase is activated by a **10 minutes, 95°C** incubation.

This PCR Master Mix is very stable, and can be stored in 4°C for up to 6 months. No need to wait the thawing.

The amplification range of the Taq is ~ 6 kb.

The PCR Master Mix contains dyes, and can directly run electrophoresis after PCR reaction.

Quality control:

This product is tested for no exogenous nuclease activity; no host DNA contamination tested (by PCR); able to amplify single copy gene from multiple genomes; and **no significant enzyme activity decrease after storing at 2~8°C for 6 months.**

Protocol

1. Prepare the reaction mix
   a. Add the desired primers and template DNA first to the PCR tube.
   b. **Gently squeeze one drop of PCR Master Mix** into the PCR tube with primers and template DNA. Then it is ready for PCR. Close the cap of the squeeze bottle after use and store at 4°C for up to 6 months.

Reagent	PCR reaction
1-Drop PCR Master Mix	1 drop
Forward Primer, 10 µM	1 µL
Reverse Primer, 10 µM	1 µL
Template DNA	≤ 4 µL

Note: 1) The volume of 1 drop PCR Master Mix is around 25 µL. It varies depending on the force of squeezing. But the variation will not affect the PCR amplification result. If larger PCR system is required, squeeze more drops of PCR Master Mix and scale up other components proportionally.

2) The recommended primer concentration for PCR is between 0.1-1.0 µM of each primer. The use of higher concentrations of primers can have higher amplification effect. Low primer concentration will generally ensure cleaner product and lower background.

3) Generally, in 25 µL PCR reaction (1 drop volume), the **DNA template** amount should be **1 pg ~ 10 ng for plasmid DNA template**; **1 ng ~ 100 ng for bacterial genomic DNA template**; **10 ng ~ 300 ng for eukaryotic genomic DNA template**. Too much template DNA may inhibit the PCR reaction.

2. PCR reaction conditions

Procedure	Temperature	Time	
Pre-denaturation	95℃	10 min	
Denaturation	94℃	30 s	30~40 cycles
Annealing	55-65℃	30 s	
Extension	72℃	60 s	
Final extension	72℃	5 min	

Note:

1) The recommended annealing temperature is about 5℃ below Tm of primers. If non-specific bands are observed, increase annealing temperature. The absence of amplification product indicates the need for a lower annealing temperature.
2) PCR extension time is depended on the size of target gene sequence. **The efficiency of *Taq* DNA polymerase in the Mix is approximately 1 kb DNA / 60 seconds.**
3) The number of PCR cycles will basically depend on the downstream application of the PCR product.

3. PCR result examination

This Master Mix contains dyes for electrophoresis. After PCR, directly load 5 µL of PCR product to agarose gel to run electrophoresis. **No need to add loading buffer.**

-- The end --