DirectTaq™ DNA Mastermix (Multiplex)

User's Instruction

Description

DirectTaq™ DNA Mastermix (Multiplex) is a PCR mastermix specially used for the direct PCR of multiple genes. After PCR, the sample can be directly loaded for electrophoresis without adding loading buffer. DirectTaq™ DNA Mastermix (Multiplex) is highly efficient, specific and sensitive, and is suitable for PCR amplification of multiple target DNA fragments (more than 20) at the same time. Moreover, this mastermix can be used not only for conventional multiplex PCR, but also for direct PCR of whole blood, serum or plant samples, which is convenient for direct multiplex PCR detection of bacterial and virus infection and gene mutation in blood samples, or multiplex PCR detection of gene mutation and microbial infection in plant samples.

Kit Contents

	100 T
1. DirectTaq™ DNA Mastermix (Multiplex) (2×)	1 ml
2. Control template and primer mix	25 μl

**The control template and primer mix contain 15 pairs of primers, which can be used as a positive control to verify and confirm the multiplex PCR amplification effect of the mastermix.*

Multiplex PCR

Multiplex PCR is a PCR technique that simultaneously amplifies at least two or more DNA fragments through a single PCR reaction. At present, this technology has been widely used in many fields, such as scientific research, disease diagnosis and forensic or diagnostic genotyping. The technology can also be used for quantitative or semi-quantitative expression analysis of genes with cDNA as template. It is especially suitable for multi gene detection of trace samples and has advantages of high specificity and high sensitivity.

Features

- **Easy operation:** After PCR, the sample can be directly loaded for electrophoresis without adding loading buffer.
- **Multiplex amplification:** The mastermix can easily realize the simultaneous amplification of at least 15 target DNA fragments.
- **High sensitivity:** For template amounts as low as 1pg, it can also be well amplified in only 30 cycles.

- **Balanced amplification:** Ordinary PCR kit may produce different amplification efficiency for different primers and templates, resulting in some fragments are easy to be amplified, while some fragments are difficult to be amplified. With high quality polymerase and optimized buffer, the balanced amplification of multiple target DNA fragments is ensured by DirectTaq™ DNA Mastermix (Multiplex).
- **High Compatibility:** Whole blood or serum samples can be directly used as templates for multiplex PCR detection of this kit without DNA extraction and purification. This mastermix is applicable to EDTA, heparin or sodium citrate anticoagulant samples or dry blood spot samples. Plant samples can also be directly used for multiplex PCR detection without DNA extraction and purification.

Protocol

Primer Design

Primer design is very important for successful multiplex PCR. We recommend to use appropriate software for primer design:

- The length of primers is usually 20-30 nucleotides.
- The GC content is preferably 45-55%.
- Avoid complementary sequences and 3 or more GC at the 3' end of primers, and avoid secondary structures in primers.
- The Tm value (Tm = 2n(A) + 2n(T) + 4n(C) + 4n(C)) of the primers used should not be lower than 60°C, preferably 65-68°C. The difference of Tm value between primers should be controlled within 5-6°C.
- The amplified target fragment should not exceed 1500bp. Although the target fragment can be amplified well when it is about 1500bp, it is relatively easy to produce bands with uneven brightness when long fragments and short fragments are amplified at the same time.
- We recommend to use high quality primers (purified by desalination, PAGE or HPLC) and dissolved in TE Buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0).

Primer Preparation

- Each primer is recommended to be prepared as 100 μm and mixed as 1:1.
- Add water to prepare primer pairs at concentration of 10 μM.

Example: *If 5' end Primer A is 18 nmol, while the 3' end Primer B is 15 nmol. Add 180 μl water or TE Buffer to Primer A to dilute to the concentration of 100 μM, and 150 μl water or TE Buffer to Primer B to dilute to the concentration of 100 μM. Take 20 μl 100 μM*

Primer A and 20 µl 100 µM Primer B into a new centrifuge tube, add 160 µl water, and mix well to obtain primer pairs (10 µM each) that can be directly used for multiplex PCR.

Setting up PCR reaction

1. Dissolve and mix solutions required for PCR reaction. Place reagents on an ice box.

2. Set up the reaction as the following table:

Reagent	Sample	Control
Nuclease-Free Water	(10-X-0.4N) µl	5 µl
DirectTaq™ DNA Mastermix (Multiplex) (2×)	10 µl	10 µl
Primer mix (10µM each)*	0.4×N µl	-
Template**	X µl	-
Control template and primer mix	-	5 µl
Total	20 µl	20 µl

**Usually the final primer concentration is recommended to be 0.2 µM or adjust between 0.05-0.4 µM according to the situation.*

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General Sample: *The amount of DNA template has a great influence on PCR amplification. For highly complex DNA samples, such as mammalian genomic DNA, we recommend to use 5ng to 0.5µg in 20 µl reaction system. For low complexity DNA, such as λDNA or plasmid DNA, we recommend to use 5pg to 5ng in 20 µl reaction system.*

Blood Sample: *When the PCR template is anticoagulant sample, the dosage of the template is generally 1-20% of the total volume of the PCR reaction system, and the recommended initial dosage is 5%. For dry blood spot samples, about 0.8 mm^2 is recommended in 20 µl reaction system.*

Plant Sample: *The recommended dosage of plant samples is 0.1-1 mm diameter leaves or other soft plant tissues of similar size in 20 µl reaction system. If use plant seeds, try to use fresh seeds with a diameter of about 0.5-2 mm without the shells.*

3. Gently blow and mix with a pipette or slightly vortex, and centrifuge at room temperature for several seconds to make the liquid volume gather at the bottom of the tube.

Thermocycling Conditions (Recommended)

1. Initial denaturation: 94°C for 5 min

2. 30-40 cycles:

a) 94°C for 30 sec

b) 60°C for 30 sec

c) 68°C 2 min/kb

3. Final extension: 68°C for 10 min

Storage

Minimum shelf life is a year at -20°C.

Only for research and not intended for treatment of humans or animals