

Direct PCR Master

Kit for direct PCR amplification from blood, animal and plant tissue

Standard PCR

	Cat. No.	Size
	PCR-111S	100 reactions x 50 µl
	PCR-111L	500 reactions x 50 µl

For *in vitro* use only

Quality guaranteed for 12 months

Store at -20°C, avoid frequent thawing and freezing

Description

Direct PCR Master is designed for PCR amplification directly from whole blood, animal tissues and plant tissues without the need of prior DNA purification processes.

The Master contains all reagents required for direct PCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The robust enzyme in combination with a specially optimized unique buffer system resists various PCR inhibitors of blood and tissue samples.

The enzyme lacks a 3'→5' exonuclease activity making this kit an ideal choice for allele specific PCR which is routinely used for various genotyping applications.

Application

- Direct PCR amplification of target DNA without any prior DNA purification step from various sample types such as whole blood, saliva, mouse tissues (tail, heart, liver, large intestine, small intestine, kidney, stomach, ear, brain, spleen), zebra fish fin, pork, beef and plant tissues (leaf and seed)
- Allele-specific PCR
- PCR for genotyping
- PCR for selection of genetically modified organisms (GMO)

Sample Preparation

Whole blood or saliva

- Add directly 1-3 µl of sample to PCR reaction mix without any pre-treatment.
Heparin, EDTA or citrate treated whole blood is suitable for this kit.

Animal or Plant tissue

- Prepare 1x DNA Extraction Buffer by diluting the provided 10x conc. Buffer with PCR-grade Water. Aliquot 50 µl of the 1x DNA Extraction Buffer into a 1.5-2 ml microtube.
- Take a small piece of tissue (about 2-3 mm in diameter) from animal or plant tissue. Plant seeds should be cracked down to a size of less than 1 mm diameter in a mortar, bead beater or tissuelyser.
- Add the tissue sample into the 1x DNA Extraction Buffer containing tube.

Direct PCR Master (red cap)

2x conc. mastermix containing polymerase, dNTPs, MgCl₂, reaction buffer and stabilizers

DNA Extraction Buffer (yellow cap)

10x conc.

PCR-grade Water (white cap)

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- Briefly mix by tapping or vortexing.
- Incubate for 3 min at RT to allow tissue lysis and DNA releasing.
- Centrifuge briefly.
- Transfer 1-3 μ l of the lysate supernatant into the PCR reaction mix.

The lysate supernatant will be stable for several weeks if stored at -20°C.

Recommended PCR assay

The preparation of a pre-mix including Direct PCR Master, primers and water is recommended to minimize pipetting errors. Dispense the pre-mix or the single components into PCR tubes or wells of a PCR plate.

component	cap	stock conc.	final conc.	20 μ l assay	50 μ l assay
Direct PCR Master	red	2x	1x	10 μ l	25 μ l
forward Primer		10 μ M	400 nM	0.8 μ l	2 μ l
reverse Primer		10 μ M	400 nM	0.8 μ l	2 μ l
Sample Preparation				1-2 μ l	1-3 μ l
PCR-grade Water	white			fill up to 20 μ l	fill up to 50 μ l

Recommended cycling conditions

Before cycling, vortex PCR tubes or plates to assure homogeneity and centrifuge briefly to remove possible bubbles.

Initial denaturation	95°C	5 min	1x
Denaturation	95°C	20 sec	35-40x
Annealing ¹⁾	45-68°C	30 sec	
Elongation ²⁾	72°C	30 sec - 4 min	
Final elongation	72°C	2 min	1x

- 1) The annealing temperature depends on the melting temperature of the primers used.
- 2) The elongation time depends on the length of the fragments to be amplified. A time of 1 min/kb is recommended.

Example: Direct Amplification from blood, saliva and plant tissues

Direct PCR Master was used to amplify a 295 bp long fragment of the beta-actin gene from whole blood and saliva and a 550 bp fragment of the Leucin tRNA gene from grass leaves and wheat seeds.

