

## Direct PCR Lyophilisate

### Lyophilized Master Mix for direct PCR amplification from blood, animal and plant tissue

	Cat. No.	Amount	Size
	PCR-160S-8TS	12 strips / 96 reactions	8-tube strips (high profile)
	PCR-160L-8TS	60 strips / 480 reactions	
	PCR-160S-FTP	2 plates / 192 reactions	96-well plates (flat top / without skirt / high profile)
	PCR-160L-FTP	10 plates / 960 reactions	
	PCR-160S-HSP	2 plates / 192 reactions	96-well plates (half skirt / high profile)
	PCR-160L-HSP	10 plates / 960 reactions	

For *in vitro* use only

Quality guaranteed for 12 months

Store below 25°C

Store in an aluminium-coated bag or on a dry place

Lyophilisates may hydrate at humidity levels >70%

when sealing is opened

#### Direct PCR Lyophilisate

Preloaded lyophilisates of Direct PCR Mastermix containing Hot Start Taq polymerase, nucleotides, optimized reaction buffer, stabilizers

#### Additives Mix (purple cap)

2 x conc.

#### DNA Extraction Buffer (yellow cap)

10 x conc.

#### PCR-grade water (white cap)

#### Description

Direct PCR Lyophilisate is delivered in PCR reaction tube strips or 96-well plates preloaded with a complete master mix in a dry, room temperature stable format. The lyophilisate combines highest performance with convenience of use and stability. There is no need for freezing, thawing or pipetting on ice. The few remaining pipetting steps minimize the risk of errors or contaminations.

To perform PCR, fill up the vials with primers, Additives Mix and PCR-grade water and add DNA template. If necessary, centrifuge to remove bubbles, vortex the vials to assure homogeneity and start cycling.

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

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 1-2 µl of sample to the PCR reaction mix without any pre-treatment.  
*Heparin, EDTA or citrate treated whole blood is suitable for this kit.*

## Direct PCR Lyophilisate

### Lyophilized Master Mix for direct PCR amplification from blood, animal and plant tissue

#### Animal or Plant tissue

- Prepare 1x DNA Extraction Buffer (yellow cap) by diluting the provided 10 x conc. Buffer with PCR-grade Water. Aliquot 20 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.
- Briefly mix by tapping or vortexing.
- Incubate for 3 min at RT to allow tissue lysis and DNA releasing.
- Centrifuge briefly.
- Transfer 1-2 µl 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 primer mix is recommended to minimize pipetting errors.

component	cap	stock conc.	final conc.	20 µl assay
Direct PCR Lyophilisate			1 x	1 tube
Additives Mix	purple	2 x	1 x	10 µl
forward Primer		10 µM	400 nM	0.8 µl
reverse Primer		10 µM	400 nM	0.8 µl
Sample Preparation				1-2 µl
PCR-grade Water	white			fill up to 20 µl

#### 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 <sup>1)</sup>	45-68°C	30 sec	
Elongation <sup>2)</sup>	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.

