



DIRECT-PCR Extraction and Amplification Kit

Fast enzymatical extraction of DNA and amplification with Hot-Start Enzyme mix

Features and Advantages:

The kit contains all components for fast extraction of DNA and the analysis by PCR. The Extraction buffer is not harmful or does not contain any irritation substances. The optimal mixture of Extraction buffers and PCR-Enzyme mix guaranties a fast and repeatable result.

- Less than 20 minutes extraction procedure

- Simple extraction step in one 0,2 ml PCR-tube – no need for washing or centrifuge equipment

- PCR Enzyme Mix includes all components even coloured loading buffer for post PCR procedure

Components:

The Direct-PCR Kit contains:

- Extraction Buffer (EXT-buffer), 10000 µl
- Thermostable Enzyme Mix (ENZ-Mix), 500 µl
- Hot-Start PCR Mix with loading dye, 1300 µl
- Sterile Water 1500 µl

Sample sources for buffer-based DNA Extraction:

Blood, swaps, animal tissue, FFPE tissue, hair follicle, insects, feathers

Transportation: on blue ice

Storage: 12 months @ -20°C

Additionally required:

0,2 ml PCR Tubes, Thermoblock, Waterbath or Thermocycler, for swaps sample 1,5 ml Tubes,

.. a good decision ..

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SAMPLE PREPARATION:

Source	Procedure	Tube size ml	µl EXT-Buffer (1X)	µl of ENZ-Mix
Blood	add 5-10 µl fresh or EDTA blood	0,2	85	5
FFPE Tissue	Trim all excessed wax from sample, 1 mm ³ or 1-2 mm ² fragment of 10 µm section FFPE tissue sample	0,2	85	5
Swaps	Cut off swap and put in	1,5		
	Dilute 1-fold EXT-buffer with water to 0.5 fold buffer (0.5X)		300 µl (0.5X)	5
Hair follicle	1-10 pcs individually follicles	0,2	85	5
Tissue from animal	2 mm ³ tissue fragment	0,2	85	5
Feathers	Place 2-5 mm quilled fragment in	0,2	100	20
Insect	Add EXT-buffer Crush insect with sterile tool	1,5	Cover all parts with x µl	5

EXTRACTION PROTOCOL:

- Close the tube with the sample
- Incubate in for
 - 10 min @ 50°C
 - 5 min @ 95 °C
- Cool down at room temperature. **Note:** You can leave the sample in the tube if not complete resolved or just remove when you have more experience with the protocol. In all cases: The extracted DNA in the Tube is safe.
- Store the DNA Extract at 4°C up to one month or continue with the PCR protocol

PCR-PROTOCOL (25µl reaction volume)

Before starting the PCR-Protocol thaw, mix and centrifuge all components in the tubes.

Components	Volume per reation
Hot-Start PCR-Mix	12,5 µl
Forward primer (10 µM stock)	0,5-1,25 µl
Reverse primer (10µM stock)	0.5-1,25 µl
Your DNA extract / Template DNA	1 µl
Sterile dest. Water (molecular grade)	up to 25 µl total reaction volume

.. a good decision ..



Note:

- vortex all solutions carefully before using
- dispense all reagents on ice
- place tubes to a Thermocycler

General Thermo-Cycler protocol:

Step	Time	Temperature
Initial denaturation	4-5 min	95°C
35-40 Cycles:		
Denaturation	15 sec	95°C
Annealing	30 sec	40-70°C
Elongation	30-60 sec	72°C per 1kb
Final Elongation	5 min	72°C

POST PCR ANALYSIS:

We recommend a 2 % Agarose gel separation. The PCR product contains two dyes (blue: about 1 kb fragment length, yellow: the front of the separation).

TROUBLE SHOOTING:

In case of:

- lysate is too thick
- or any problem with pipetting: centrifuge and uses supernatant in PCR-Reaction; or dilute the lysate 1:5 to 1:10 with TE buffer or Tris-HCl, pH 8.0.
- of non-specific or lack of PCR product dilute the lysate 1:5 to 1:10 with TE buffer or Tris-HCl, pH 8.0.

Cat.-no	Description	Amount
S700	DIRECT-PCR Extraction and Amplification Kit	100 rcs
S700L	DIRECT-PCR Extraction and Amplification Kit	5x100 rcs
S700XL	DIRECT-PCR Extraction and Amplification Kit	20x100 rcs

Safety informaton

ENZ-Mix

H315 Causes skin irritation.

H319 Causes serious eye irritation.

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

H335 May cause respiratory irritation.

P261 Avoid breathing dust.

P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P342+P311 If experiencing respiratory symptoms call a Poison Center or doctor /physician.



DANGER

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