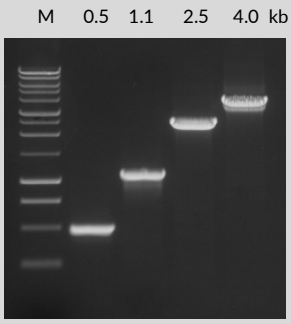
EURx® MOLECULAR BIOLOGY PRODUCTS

Direct Tissue PCR Kit

Cat. No.	size
E0940-01	100 reactions of 50 µl
E0940-02	500 reactions of 50 µl

Storage Conditions: Store at -20°C.



PCR amplification using EURx Direct Tissue PCR Kit.

Lane M: molecular size marker – Perfect Plus 1 kb DNA Ladder (E3131).

Lanes 0.5 to 4 kb: PCR amplification reactions directly from human liver, using the Extract protocol, human-specific primers and EURx Direct Tissue PCR Kit.

Direct Tissue PCR Kit enables to perform PCR reactions directly from tissue samples with no prior DNA purification.

Description:

- The Direct Tissue PCR Kit allows to use samples such as: animal tisues, mouse ear and tail, zebrafish fin, Drosophila, human hair, saliva and other liquid samples.
- The samples can either be fresh or stored at -20°C.
- The Direct Tissue PCR Kit employs a hot start and genetically engineered thermophilic DNA polymerase with high tolerance to blood inhibitors.
- Tissue DNA Polymerase activity is restored during 7 min initial denaturation step.
- Tissue DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction.
- The enzyme exhibits the 3'→5' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.
- The enzyme generates blunt ends.
- Enhanced polymerase processivity allows to use shorter extension times.
- **Due to the genetic modification of the polymerase, the optimal reaction conditions (especially annealing temperatures) differ from standard PCR protocols**.
- The Direct Tissue PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols.
- The master mix contains premixed gel loading reagent and dyes which allow direct sample loading on the gel.
- The Direct Tissue PCR Kit allows to obtain a wide range of product size (over 4 kb).

Direct Tissue PCR Kit contains:

1. 2 x Tissue PCR Master Mix
2. Tissue DNA Polymerase
3. Extraction Buffer
4. Lysis Enhancer
5. Water, nuclease free

2 x Tissue PCR Master Mix:

The master mix contains 2 x concentrated optimized PCR buffer, 5 mM $MgCl_2$, dNTPs and two gel tracking dyes.

Tissue DNA Polymerase Storage Buffer:

20 mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.1 mM EDTA, 1 mM dithiothreitol, 50% glycerol and stabilizers.

Quality Control:

All preparations are assayed for contaminating 3'-exonuclease, and nonspecific single - and double-stranded DNase activities. Typical preparations are greater than 95% pure, as judged by SDS polyacrylamide gel electrophoresis.

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Sample handling

To obtain small and uniform samples, we recommend using 0.35-0.5 mm diameter puncher or scalpel. If the puncher or scalpel is to be reused, it is important to clean the cutting edge properly to prevent cross-contamination between samples. Use 2% NaClO solution for cleaning and cross-contamination prevention.

Choosing the protocol

The Direct Tissue PCR Kit contains reagents for two alternative protocols: Direct and Extract protocols. With a few exceptions, both Direct and Extract protocol are compatible with all sample types and application.

The Extract protocol is recommended:

- When working with new sample materials or a new primer pair.
- With difficult or long amplicons (over 1 kb).
- When performing multiple reactions from the same sample.

For Extract protocol use 20-50 µl reaction volume and for Direct protocol use only 50 µl reaction volume.

The samples in Extraction Buffer can be stored for up to 4 weeks at 4°C or -20°C before using in PCR.

Solid samples

Direct protocol

Take a sample:

- 0.35-0.5 mm in diameter from animal tissue,
- 1-3 hair bulbs,
- 1 x 1 mm nail sample,
- 1 x 1 mm teeth sample.

Place the sample directly into a 50 µl PCR reaction. Perform PCR (see the table on the next page).

After PCR, add 1.5 µl of Lysis Enhancer to a 50 µl of PCR reaction, incubate for 5 min and centrifuge the reaction at maximum speed for 1 min to pellet debris from the tissue samples.

Extract protocol

Place the tissue sample into 20 µl of Extraction Buffer. Add 0.5 µl of Lysis Enhancer. If a larger sample is used, adjust the volume of the Extracion Buffer and Lysis Enhancer accordingly. Mix the tube briefly and spin down the solution. Incubate the reaction for 5 minutes at room temperature followed by 5 min at 98°C. Spin down again and store the supernatant at 4°C or –20°C. Use 0.5-1 µl of the supernatant as a template in a 20 µl PCR reaction.

Liquid samples

Direct protocol

Add 0.5-1 µl of the liquid sample (saliva, amniotic fluid) directly to a 50 µl of PCR reaction. Perform PCR (see the table on the next page).

After PCR, add 1.5 µl of Lysis Enhancer to a 50 µl of PCR reaction, incubate for 5 min and centrifuge the reactions at maximum speed for 1 min to pellet debris from the tissue samples.

Extract protocol

Add 5 µl of the liquid sample to the tube that contains 20 µl of Dilution Buffer and 0.5 µl of Lysis Enhancer. Mix by vortexing and spin down. Incubate the reaction for 5 minutes at room temperature followed by 5 min at 98°C. Spin down again and store the supernatant at 4°C or –20°C. Use 0.5-1 µl of the supernatant as a template in a 20 µl PCR reaction.

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Preparation of PCR Reaction From Tissue Sample:

Component	20 µl reaction	50 µl reaction	Final concentration
2 x Tissue PCR Master Mix	10 µl	25 µl	1x 2.5 mM $MgCl_2$
Primer A	Variable	Variable	0.5 µM
Primer B	Variable	Variable	0.5 µM
Tissue DNA Polymerase	0.4 µl	1 µl	
Sample Direct protocol Extract protocol	- 0.5-1 µl	0.35-0.5 mm tissue sample 2.5 µl	
Sterile double-distilled water	Add to 20 µl	Add to 50 µl	
Total volume	20 µl	50 µl	

Notes:

1. Completely thaw and mix thoroughly all components of PCR reaction before use to avoid localized differences in salt concentration.
2. Prepare reaction mixes at room temperature. Use of Tissue DNA Polymerase allows room temperature reaction setup. Mix well.
3. Reactions can be placed in a room temperature thermal cycler.
4. The 2 x Tissue PCR Master Mix allows PCR reactions to be loaded directly onto an agarose gel without prior addition of a gel loading buffer. The master mix contains a gel loading reagent and two gel tracking dyes (a red dye and a yellow dye) that separate during electrophoresis. In a 1% agarose gel, the red dye migrates at the same rate as 600 bp DNA fragment and the yellow dye migrates faster than 20 bp. The dyes do not interfere with most downstream enzymatic applications, however it is recommended to purify PCR products prior enzymatic manipulation.
5. In most cases there is no need to add additives to PCR reaction. For some difficult targets such as: GC-rich sequences, sequences with complex secondary structures additives such as DMSO can be included to improve amplification. The recommended starting DMSO concentration is 3% (if needed).

Thermal Cycling Conditions:

Step	2-step protocol		3-step protocol		Number of Cycles
	Temp.	Time	Temp.	Time	
Lysis of Cells, Initial Denaturation	98°C	7 min	98°C	7 min	1
Denaturation	98°C	5-10 s	98°C	5-10 s	35-40
Annealing	-	-	X°C	15-30 s	
Extension	72°C	30 s/1 kb	72°C	30 s/1 kb	
Final Extension	72°C	1 min	72°C	1 min	1
Cooling	4°C	Indefinite	4°C	Indefinite	1

Notes:

1. A 7-minute initial denaturation at 98°C enables lysis of cells and Tissue DNA Polymerase activation.
2. Tissue DNA Polymerase has the ability to stabilize primer-template hybridization. Melting temperatures (T_m) and optimal annealing temperatures usually differ significantly (are higher) from the temperatures calculated/determined for standard PCR polymerases. T_m's should be calculated with the base-stacking method (nearest-neighbor method). Use the calculator of the base-stacking method on EURx's website (www.eurx.com.pl). Default parameters are: 500 nM primer concentration, 50 mM salt concentration, 1.5 mM Mg^{2+} concentration. As a basic rule use an annealing temperature at a T_m of the lower T_m primer. In some cases optimal annealing temperatures may differ from the rule given above and should be determined empirically.
3. A 2-step protocol allows to perform combined annealing/extension step at 72°C and is recommended for primers with T_m values of at least 72°C. The 2-step protocol allows to save time of PCR reaction.
4. Extension time of 30 s/ 1 kb is recommended for most targets.

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