

CERTIFICATE OF ANALYSIS
Fast Tissue-to-PCR Kit
#K1091 (for 200 reactions)

Lot: ___ Expiry Date: ___

Store at -20°C.


Components of the kit

Component	#K1091 200 rxns
Tissue Lysis Solution	50 ml
Proteinase K	2x1 ml
Neutralization Solution T	50 ml
Tissue Green PCR Master Mix (2X)	2x1.25 ml
Water, nuclease-free	2x1.25 ml

Description

The Fast Tissue-to-PCR Kit is a complete system for rapid extraction and amplification of genomic DNA from a variety of tissue samples. The kit can be used to extract and amplify DNA from mouse tail and ear clips, animal tissues, human hair shafts or saliva, fish and insect tissues, and buccal swabs. There is no need for mechanical disruption of tissues, overnight digestion, phenol extraction, DNA precipitation or column purification.

The procedure begins with a 10 minute incubation of the sample in Tissue Lysis Solution supplemented with Proteinase K at room temperature. Neutralization Solution T is then added, and the tissue extract is ready for PCR with the Tissue Green PCR Master Mix (2X).

The PCR Master Mix includes a specialized formulation of the highly robust and efficient DreamTaq™ DNA polymerase, dNTPs and buffer optimized for use with the extraction reagents provided in the kit. Loading dyes and a density reagent are also included in the master mix for direct loading of the PCR product onto an agarose gel.

The Tissue Green PCR Master Mix (2X) is ideal for amplifying targets up to 2 kb and multiplex PCR with up to 4 primer pairs. The PCR product can be directly used in downstream applications such as DNA digestion and sequencing without further purification.

Applications

- Genotyping
- Transgene detection
- Knockout analysis
- Sequencing

PROCEDURE

Important Notes

- Rinse all tools (scissors, scalpel) with 70% ethanol.
- Extraction step is carried out at room temperature.
- PCR reaction is assembled on ice.

I. DNA extraction

DNA extraction from tissues or saliva

Recommended sample sizes per prep

- 0.3-0.5 cm mouse tail
- 0.5 -2 mm mouse ear punch
- 2-5 mg piece of tissue
- 1-10 hairs with roots
- 20 µl saliva
- 2-3 mm² piece of zebrafish fin

1. Pipette 100 µl of Tissue Lysis Solution into a microcentrifuge tube or plate well, add 10 µl of Proteinase K Solution and mix by vortexing.

Note. For multiple extractions, sufficient volumes of Lysis and Proteinase K Solutions may be premixed at a ratio of 10:1 up to 2 hours before use.

2. Add the recommended amount of sample to the tube or plate well containing the prepared lysis mix. Ensure that the sample is fully submerged in the solution.

3. Incubate the sample at room temperature for 10 minutes.

Note. For smaller sample sizes, incubation at 55°C may enhance tissue lysis and amplification.

4. Incubate the sample at 95°C for 3 minutes.

Note. At the end of the incubations, tissues may not be completely digested - this does not affect PCR performance.

5. Add 100 µl of Neutralization Solution T to the sample and mix by vortexing.

6. Store the neutralized tissue extract at 4°C or use immediately in PCR.

Note. For long term storage at 4°C remove the undigested tissue or transfer the extracts to new tubes or wells.

DNA extraction from Buccal Swabs

1. Collect buccal cells on swab (a foam-tipped swab is recommended) and dry the swab at room temperature for about 10 minutes.
2. Pipette 200 µl of Tissue Lysis Solution into a microcentrifuge tube or plate well, add 10 µl of Proteinase K Solution and mix by vortexing.

Note. For multiple extractions, sufficient volumes of Lysis and Proteinase K Solutions may be premixed at a ratio of 20:1 up to 2 hours before use.

3. Place the dried buccal swab into the prepared lysis solution for 2 minutes and rotate the swab in the solution at least 5 times.
4. Rotate and press the swab firmly against the side of the tube to ensure that most of the liquid remains in the tube. Discard the swab.
5. Incubate the sample at room temperature for 5 minutes.
6. Incubate the sample at 95°C for 3 minutes.
7. Add 200 µl of Neutralization Solution T to the sample and mix by vortexing.
8. Store the neutralized tissue extract at 4°C or use immediately in PCR.

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II. PCR amplification

- Gently vortex and briefly centrifuge the Tissue Green PCR Master Mix (2X) after thawing. Place a thin-walled PCR tube or plate on ice and add the following components for each 20 μ l reaction:

Water, nuclease-free (#R0581)	to 20 μ l
Tissue Green PCR Master Mix (2X)	10 μ l
Forward primer	0.1- 1 μ M
Reverse primer	0.1- 1 μ M
Tissue extract	4 μ l
Total volume	20 μl

- Gently vortex and briefly centrifuge.
Optional. When using a thermal cycler that does not use a heated lid, overlay the reaction mixture with 10 μ l of mineral oil.
- Perform PCR using the following thermal cycling conditions:

Step	Temperature, $^{\circ}$ C	Time	Number of cycles
Initial denaturation	95	1-3 min	1
Denaturation	95	30 s	30-40
Annealing	Tm-5	30 s	
Extension	72	1 min/kb	
Final Extension	72	3 min	1
Hold	4	∞	

- Load 5-15 μ l of the PCR mixture directly on a gel. There is no need to add loading dye.
PCR products can be used directly for a variety of applications. The presence of loading dyes does not interfere with DNA sequencing and digestion. Alternatively, PCR products can be purified using spin column, e.g. GeneJET™ PCR Purification Kit (#K0702).


TROUBLESHOOTING

Problem	Cause and Solution
Low yield or no PCR product	PCR inhibition due to contaminants in the tissue extract. Dilute the tissue extract with nuclease-free water.
	Insufficient DNA extraction. Incubate samples at 50-55 $^{\circ}$ C for 10 minutes instead of incubation at room temperature.
	Insufficient number of cycles performed. Generally 30-35 cycles are sufficient; however increased number of cycles may produce better results in some cases.
	Faulty primer design. Use primer design software. Ensure primers are not self-complementary and there are no complementary sequences between primers. Verify that the primers are complementary to the correct strands of template DNA
Non-specific PCR products	Suboptimal annealing temperature. Decrease the annealing temperature in 2-4 $^{\circ}$ C increments
	PCR reaction set up at room temperature. PCR reaction set up should be performed on ice.
PCR product in negative control	Suboptimal annealing temperature. The optimal annealing temperature is normally about 5 $^{\circ}$ C lower than the primer-template melting temperature (Tm). It may be optimized stepwise in 1-2 $^{\circ}$ C increments.
	Cross contamination. PCR was contaminated by DNA present in the working environment. Avoid contamination by following general recommendations for PCR preparation.

QUALITY CONTROL

The absence of endo-, exodeoxyribonucleases and nicking activity in components is confirmed by appropriate quality tests.

All kit components are functionally tested in multiplex PCR amplification of 114, 214, 388 and 515 bp fragments from mouse 2 mm ear punch lysate.

Quality authorized by:  Jurgita Zilinskiene

SAFETY INFORMATION



Proteinase K

Xn Harmful

Hazard-determining components of labeling: Proteinase, Tritirachium album serine

Risk phrases

R42 May cause sensitization by inhalation.

Safety phrases

S23 Do not breathe gas/fumes/vapor/spray.

S36 Wear suitable protective clothing.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

S60 This material and its container must be disposed of as hazardous waste.

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