

AMPQX® Animal Tissue Direct PCR Kit -with Dye

Cat. no. LM201012-3

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1. Product Information

Product name	AMPQX® Animal Tissue Direct PCR Kit -with Dye
Cat. No.	LM201012-3
Size	50T/200T
Storage and shipping	1.[A] For animal tissue lysis buffer, recommended to save at 2 - 8°C 2.[B] For animal tissue cracking agent, do not open the lid for a long time when using. It is recommended to keep it at -20 °C to avoid repeated freezing and thawing. 3.[C] 2× Tissue Direct PCR Mix (With Dye). It is recommended to keep it at -20°C to avoid repeated freezing and thawing. 4.[D] 5× PCR Enhancer .It is recommended to keep it at -20 °C to avoid repeated freezing and thawing.

2. Product description

Animal tissue direct PCR kit is a kit that can directly conduct PCR amplification of different animal tissues, wide adaptability, strong stability, easy to operate. This kit uses a unique lysis buffer system, which can quickly break a variety of animal tissue samples and release genomic DNA, such as insect foot wings, mouse tail, mouse toe, animal skin and internal organs. The cleavage products can be used for DNA extraction and purification, or directly for PCR reactions, or for long-term storage at temperatures of -20°C or below.

The 2× Tissue Direct PCR Mix (With Dye) provided in this kit has strong amplification compatibility, which does not require additional purification extraction reagent to remove various debris impurities in the lysis product, and can directly use the sample lysate as template for high efficiency and specific amplification. This reagent is a 2× concentrated PCR reaction mixture and contains all components used for PCR amplification except the template and primer, greatly simplifying the operation of the amplification step and reducing the contamination rate. This kit can be used for animal gene amplification detection and genotype identification of transgenic animals.

3.Components

Compont No.	Name	Format	
		50T	200T
A	Lysis Buffer	10 mL	20×2 mL
B	Proteases	100 µL	400 µL
C	2× Tissue Direct PCR Mix (With Dye)	500 µL	1×2 mL
D	5× PCR Enhancer	200 µL	800 µL

- a) Lysis Buffer and Proteases were used in animal tissue lysis
- b) 2× Tissue Direct PCR Mix (With Dye) contains the hot start Taq DNA polymerase, dNTP and Mg2+ required for PCR amplification; bromophenol blue dye as an electrophoresis indicator and the PCR product can be directly subjected to electrophoresis.
- c) 5× PCR Enhancer: 5× PCR Enhancer is recommended for high GC amplification (above 65%).

4.Operate

1. Add 200 µL Lysis Buffer and 2 µL Proteases in the centrifuge tube and mix gently
2. Place about 10 mg (about 2 mm in length) in the above centrifuge tube and mix gently.
3. The cells were incubated at 55°C for 5 – 30 min and then 95°C for 5 min.
4. Was centrifuged at 12000 rpm for 2 min.
5. The supernatant was transferred to a new centrifuge tube and stored at -20°C or used directly for PCR amplification.

[Note]:

- a) Lysis Buffer Mix with Proteases as soon as possible, not save for long time. For a large number of samples, Lysis Buffer and Proteases can be mixed in the ratio of 100 µL: 1 µL for reserve.
- b) Tissue should be small and cut as much as possible so that the lysis reaction can proceed more smoothly. Recommended usage for each organization: rat tail: 2-4 mm in length; rat toe: 1-4; rat organs, brain: 2-4 mm in diameter; zebrafish, nematodes, fruit flies and other insect tissues: 1-4; cell number: 105-108. Tissues from smaller samples such as zebrafish, nematodes, and Drosophila can appropriately reduce Lysis Buffer to 50-100 µ L. For samples with hard shells such as insects, it is recommended to cut the samples and add Proteases to 4 µL.
- c) With 55°C incubation, generally 5 min can meet most PCR requirements, such as mouse tail, mouse ear and other tissues; if the amount of DNA required is large or the sample is difficult to crack, the time can be extended to 30 min or more; the lysis time can be adjusted between 5-30 min, the tissue block does not need to be completely cracked, and the residual part can be removed in the subsequent centrifugation step.

4.1. PCR reaction System

Components	Volume μ L	Final Conc.
2 $\times$ Tissue Direct PCR Mix(With Dye)	10	1 $\times$
Forward Primer (10 μ mol/L)	0.5	0.25 μ mol/L
Reverse Primer (10 μ mol/L)	0.5	0.25 μ mol/L
Template	2	--
ddH ₂ O	Up to 20	

[Note]: All components should be thoroughly mixed before use.

a) Template usage: it is recommended to be between 5-20% of the total system and avoid exceeding 20% of the total system.

b) Primer final concentration: When the reaction performance is poor, it is recommended to adjust the primer concentration in the range of 0.1-0.5 μ M.

c) Reaction system: 20 μ L is recommended to ensure the validity and reproducibility of the target gene amplification.

d) System preparation: prepare the PCR reaction system, vortex and mix evenly on the vortex meter, and collect the reaction solution at the bottom of the tube by instantaneous centrifugation.

e) Control reaction: It is recommended that during PCR, set up positive and negative PCR control reactions to exclude false positive or false negative interference

4.2. Reaction program

Cycle step	Temp.	Time	Cycles
Initial denaturation	94 $^{\circ}$ C	5 min	1
Denaturation	94 $^{\circ}$ C	10 sec	35
Annealing	60 $^{\circ}$ C	20 sec	
Extension	72 $^{\circ}$ C	20 sec	
Final extension	72 $^{\circ}$ C	5 min	1

[Note]:

1. Annealing temperature: please refer to the theoretical T_m value of the primer. The annealing temperature can be set 2-5 $^{\circ}$ C lower than the theoretical value of the primer.

2. Extension time: It needs to be determined according to the length of the fragment. For DNA fragments within 1 kb, the recommended extension time is 1 min.

5. Notes

1. For your safety and health, please wear lab coats and disposable gloves for operation.

2. This product is for research use ONLY !