HB220429

Animal Tissue Direct PCR Kit (with Dye)

Product Information

Product name	Cat#	Size
Animal Tissue Direct PCR Kit (with Dye)	10184ES50	50 T
	10184ES70	200 T

Product Description

Animal Tissue Direct PCR Kit is a kit that designed for PCR amplification directly from different animal tissues, with wide adaptability, strong stability and easy operation.

The kit uses a unique lysis buffer system, which can lyse a variety of animal tissue samples quickly and then release genomic DNA, such as insect foot wings, mouse tails, mouse toes, animal skin and internal organs. The lysate can be used for DNA extraction and purification, PCR reactions, or stored at -20°C or below for long-term use.

In the kit, the 2× Tissue Direct PCR Mix (With Dye) is highly compatible with amplification. There is no need to remove various debris impurities using additional purification and extraction reagents in the lysate. It directly uses the lysate of the sample to be tested as a template for amplification efficiently and specifically. The Animal Tissue Direct PCR Kit includes PCR reaction mixture (2×), a ready-to-use PCR mix comprising all the components for PCR amplification, except primers and template, which simplifies the operation of the amplification step and reduces the probability of contamination greatly.

The kit can be used for animal gene amplification detection and transgenic animal genotype identification.

Product Components

Component number	Components	Cat#/Size	
		10184ES50 (50 T)	10184ES70 (200 T)
10184-A	Lysis Buffer	10 mL	20×2 mL
10184-B	Proteases	100 μL	400 μL
10184-C	2× Tissue Direct PCR Mix (with Dye)	500 μL	1×2 mL
10184-D	5× PCR Enhancer	200 μL	800 μL

a) The Lysis Buffer and Proteases are used in combination for animal tissue lysis.

b) 2× Tissue Direct PCR Mix (with Dye) contains hot-start Taq DNA polymerase, dNTPs and Mg^{2+} required for PCR amplification. It has been mixed with bromophenol blue dye as an electrophoresis indicator, and the PCR product can be electrophoresed directly.

c) 5× PCR Enhancer: 5× PCR Enhancer is recommended for high GC content amplification (such as greater than 65 %).

Shipping

The components are shipped with ice packs and can be stored for 1 year.

Storage

1. Reagent 10184-A [Lysis Buffer] is animal tissue lysis buffer, it is recommended to store at 2-8℃.

2. Reagent 10184-B [Proteases] is an animal tissue lysing agent. Do not open the cap for a long time when using it. It is recommended to store at -20°C to avoid repeated freezing and thawing.

3. Reagent 10184-C [2× Tissue Direct PCR Mix (With Dye)], it is recommended to store at -20℃ to avoid repeated freezing and thawing.

4. Reagent 10184-D [5× PCR Enhancer], it is recommended to store at -20°C to avoid repeated freezing and thawing.

Method of application

1. Add 200 μL Lysis Buffer and 2 μL Proteases to a centrifuge tube and mix by vortexing gently.

2. Take about 10 mg (about 2 mm in length) of animal tissue, put it in the above centrifuge tube, and mix by vortexing gently.

3. Incubate at 55°C for 5-30 min, then treat at 95°C for 5 min.

4. Centrifuge at 12,000 rpm for 2 min.

5. Transfer the supernatant to a new centrifuge tube and store at -20°C or use for PCR amplification directly.

[Note]:

a) Lysis Buffer and Proteases should be used as soon as possible after mixing. The mixture cannot be stored for a long time. When handling a large number of samples, Lysis Buffer and Proteases can be mixed at a ratio of 100 μL: 1 μL for use.

b) A small amount of tissue should be taken and chopped as much as possible, so that the lysis reaction can proceed more smoothly. Recommended dosage for each tissue: mouse tail: 2-4 mm in length; mouse toe: 1-4; mouse organs and brain: 2-4 mm in diameter; the number of zebrafish, nematodes, fruit flies or other insect tissues: 1-4; number of cells: 10^5-10^8 cells. For tissues from smaller samples such as zebrafish, nematodes, and Drosophila, the Lysis Buffer can be appropriately reduced to 50-100 μL. For samples with hard shells, such as insects, it is recommended to chop the sample and add Proteases to 4 μL.

c), It can meet most PCR requirements to incubation at 55°C and 5 mins generally, such as mouse tail, mouse ear and other tissues. If the amount of DNA required is large or the sample is difficult to lyse, the lysis time can be extended to 30 minutes or longer. The lysis time can be adjusted between 5-30 min, the tissue block does not need to be completely lysed, and the residual part can be removed in the subsequent centrifugation step.

PCR Reaction System

Components	Volume (μL)	Final Concentration
2× Tissue Direct PCR Mix (with Dye)	10	1×
Forward Primer (10 μmol/L)	0.5	0.25 μmol/L
Reverse Primer (10 μmol/L)	0.5	0.25 μmol/L
Lysate (DNA template)	2	-
PCR-grade water	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, avoid exceeding 20% of the total system.

b) Final primer concentration: it is recommended to adjust the primer concentration in the range of 0.1-0.5 μmol/L, when the reaction performance is poor.

c) Reaction system: the volume of rection system is recommended for 20 μL to ensure the validity and repeatability of target gene amplification.

d) System preparation: Prepare the PCR reaction system, then place it on a vortexer, vortex and mix, and centrifuge briefly to collect the reaction solution at the bottom of the tube.

e) Control reaction: it is recommended to set positive and negative PCR control reactions when performing PCR in order to exclude false positive or false negative interference.

PCR reaction protocol

Cycle step	Temp.	Duration	Cycles
Initial denaturation	94℃	5 min	1
Denaturation	94℃	10 sec	35
Annealing*	60℃	20 sec	
Extension	72℃	20 sec	
Final extension	72℃	5 min	1

*Annealing temperature: Please refer to the theoretical Tm value of the primer. The annealing temperature can be set 5°C lower than the theoretical value of the primer. The optimal temperature can be determined by gradient PCR.

Cautions

1. It is recommended to use freshly collected animal tissues. If the tissues are frozen for a long time, repeated freezing and thawing should be avoided as much as possible, otherwise the template will be degraded and the PCR efficiency will be affected.
2. For your safety and health, please wear lab coats and disposable gloves for operation.
3. This product is for research use **ONLY**!