TransDirect® Animal Tissue PCR Kit

Please read the manual carefully before use.

Cat. No. AD201

Storage: at -20°C for two years

Description

TransDirect® Animal Tissue PCR Kit uses a unique lysis buffer to lyse tissues (fresh or frozen). The resulting lysate without purification can be directly used as PCR template. 2×*TransDirect*® PCR SuperMix (+dye) is highly resistant to various PCR inhibitors present in animal tissues. PCR product can be directly used for gel electrophoresis. AD3 Buffer is recommended to use in aliquots.

Applications

• Direct amplification from unpurified lysate. Suitable for high throughput screening.
• Suitable for mammalian cells, blood, saliva, hair and animal tissues (mammals, marine animals, insects) etc.
• Amplification of genomic DNA fragment up to 3 kb.

Kit Contents

Component	AD201-01	AD201-02
AD1 Buffer	4 ml	20 ml
AD2 Buffer	1 ml	5 ml
AD3 Buffer	4 ml	2×10 ml
2×*TransDirect*® PCR SuperMix (+dye)	1 ml	5×1 ml
Nuclease-free Water	5 ml	25 ml

Sample Requirements

Material	Amount
Mammalian Cells	≤10^6 cells
Hair	≤10 mg
Animal Tissues	≤10 mg
Mouse Ear	≤0.5 cm^2
Mouse Tail	≤0.5 cm
Saliva	≤10 µl
Blood	≤10 µl

Procedures

Prepare a 95°C water bath or metal bath in advance.

A. Sample Lysis

1. Mix 40 µl of AD1 buffer with 10 µl of AD2 buffer. For more samples, premix AD1 buffer with AD2 buffer at a ratio of 4:1. The mixture can be stored up to 2 hours at room temperature.
2. Different samples, the treatment method is as follows:

a.Mammalian Cells

Completely remove the culture medium from the collected cells. Add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.

b.Animal Tissues

Cut up tissues with sterile scissors or blade, add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.

c.Saliva

Directly add saliva into the mixture of AD1/AD2, mix thoroughly by pipetting up and down.

d.Hair

Cut hair into pieces, add the mixture of AD1/AD2, mix thoroughly by pipetting up and down.

e.Blood
Directly add blood into the mixture of AD1/AD2, mix thoroughly by pipetting up and down.
3. Incubate at room temperature for 10 minutes, followed by at 95°C for 3 minutes (for hard-to-lyse tissues, like hair, we suggest incubating at 55°C for 10 minutes, followed by at 95°C for 3 minutes).
4. Add 40 µl of AD3 buffer, mix well. The lysate can be used as PCR template or stored at 2-8°C for three months or at -20°C for six months.
B. PCR
Reaction Components (20 µl)

Component	Volume	Final Concentration
Unpurified Lysate	Variable (≤4 µl)	as required
Forward Primer (10 µM)	0.4 µl	0.2 µM
Reverse Primer (10 µM)	0.4 µl	0.2 µM
2×*TransDirect*® PCR SuperMix (+dye)	10 µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

PCR

94°C	5-10 min	
94°C	30 sec	35-40 cycles
50-60°C	30 sec	
72°C	1-2 kb/min	
72°C	5-10 min	

Notes
- Samples avoid repeated freezing and thawing.
- Completely thaw the contents in the tube and mix well before use.
- For tissue that is not easy to lysis (such as hair), it can be incubated at 55°C for 10 minutes.
- If faint bands are observed, use more PCR template or increase the number of PCR cycles (no more than 40 cycles). If non-specific amplification bands are observed, adjust the annealing temperature or properly reduce the quantity of template used.
- The extracts can be stored at 2-8°C for three months or at -20°C for six months.