TransDirect™ Animal Tissue PCR Kit

Cat. No. AD201

Storage: at -20°C for two years

Description

TransDirect™ Animal Tissue PCR Kit uses a unique lysis buffer to lyse animal tissues (fresh or frozen) and blood. Resulting lysate without purification can be directed used as template for PCR amplification. 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. It is recommended to aliquot AD3 buffer before use.

Applications

- Direct amplification from unpurified lysate. Suitable for high throughput applications.
- Suitable for mammalian cells, saliva, hair shaft, animal tissues and blood.
- Amplification of genomic DNA fragment up to 3 kb.

Kit Contents

Component	AD201-01	AD201-02
AD1 Buffer	12 ml	55 ml
AD2 Buffer	3 ml	15 ml
AD3 Buffer	12 ml	5×12 ml
2×*TransDirect*™ PCR SuperMix (+dye)	1 ml	5×1 ml
ddH_2O	5 ml	25 ml

Amount of Starting Material

Material	Amount
Mammalian Cells	1-5×10^6 cell
Animal Tissues	10-30 mg
Mouse Tail	0.5-1 cm sections
Mouse Ear	0.5-0.7 cm disk
Saliva	10-30 µl
Hair shaft	30 mg
Blood	20 µl

1. Genomic DNA extraction

   Mix 100 µl of AD1 buffer with 25 µl of AD2 buffer. For more samples, premix AD1 buffer with AD2 buffer at ratio of 4:1. The mixture can be stored up to 2 hours at room temperature.

2. Treatment method for different samples

- Mammalian Cells

  Pellet the cells by centrifugation. Remove the supernatant. Add the mixture of AD1 and AD2, mix thoroughly by pipetting up and down.

- Saliva

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

- Hair Shafts

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

- Animal Tissues

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

- Blood

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

3. Incubate at room temperature for 10 min, followed by at 95°C for 3 minutes (For cells hard to be lysed, like hair , suggest to incubate at 55°C for 10 minutes, followed by at 95°C for 3 minutes).
4. Add 100 µl of AD3 buffer, mix well. The lysate can be used as PCR template or stored at 4°C or at -20°C.

Reaction Components

Component	Volume	Final Concentration
Tissue Extract	4 µl	as required
Forward Primer (10 µM)	0.4-0.8 µl	0.2-0.4 µM
Reverse Primer (10 µM)	0.4-0.8 µl	0.2-0.4 µM
2×*TransDirect*™ PCR SuperMix (+dye)	10 µl	1×
ddH_2O	Variable	-
Total volume	20 µl	-

Thermal cycling conditions

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

- Avoid repeated freezing and thawing of samples.
- Completely thaw the contents in the tube and mix well before use.
- If faint bands are observed, increase the quantity of template used 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 4°C for three months or at -20°C for six months.

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