

TransDirect[®] Animal Tissue PCR Kit

Please read the manual carefully before use.

Cat. No. AD201

Version No. Version 2.0

Storage: at -18°C or below for two years

Description

This kit uses a unique lysis buffer to lyse tissue samples (fresh or frozen tissue). The resulting lysate can be used directly as a template without the need for purification and is amplified using the inhibitor-resistant 2×TransDirect[®] PCR SuperMix II (+dye). The amplified products can be loaded directly for electrophoresis. It is recommended to aliquot AD3 Buffer for use.

Features

- 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 II (+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, metal bath, or PCR instrument in advance.

A: Sample Lysis

1. Add 10 μl of AD2 Buffer into 40 μl of AD1 Buffer and mix by pipetting up and down. (If processing many samples, you may pre-mix AD1 Buffer and AD2 Buffer at a 4:1 ratio in advance, and use within two hours.)

2. For different sample types, proceed as follows:

a. Animal Cells

After collecting the cells, completely remove the culture medium, add the AD1 and AD2 mixture, and mix by pipetting up and down.

b. Animal Tissue

Mince the tissue with clean, sterile scissors or a blade, then add the AD1 and AD2 mixture and mix by pipetting up and down.

c. Saliva

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

d. Hair

After cutting the collected hair into fine pieces as much as possible, add it into the AD1 and AD2 mixture and mix by pipetting up and down.



e. Blood

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

3. Incubate at 55 °C for 10 minutes, then incubate at 95 °C for 3 minutes.

4. Add 40 µl of AD3 Buffer, mix well, and use the mixture directly as a template for PCR, or store at 2–8 °C or –20 °C.

Reaction Components (taking 20 µl reaction system as an example)

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× <i>TransDirect</i> [®] PCR SuperMix II (+dye)	10 µl	1×
Nuclease-free Water	Variable	-
Total volume	20 µl	-

PCR

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

Note: The annealing temperature X is the theoretical T_m value of the primers ±2–5 °C.

Notes

- Avoid repeated freezing and thawing of samples.
- Ensure reagents are completely thawed and thoroughly mixed before use.
- Primer concentration is typically 0.2 µM, but can be adjusted within the range of 0.1–0.5 µM based on experimental results.
- The optimal template amount is generally no more than 10% of the total reaction volume. For a 20 µl system, the recommended template volume is 1–2 µl, and should not exceed 4 µl, as excessive template may inhibit the PCR reaction.
- If amplification bands are weak, consider appropriately increasing the template amount or the number of PCR cycles (not exceeding 40 cycles). If non specific amplification is observed, adjust the annealing temperature or modify the template amount.
- Lysates can be stored at 2–8 °C for up to three months and at –20 °C for up to six months.

For research use only, not for clinical diagnosis.

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Service telephone +86-10-57815020

Service email custserv@transgenbiotech.com

