KiaDirect™ Animal Tissue PCR Kit

Cat. No. AD201
Storage: at -20°C for two years

Description

KiaDirect 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. *2xKiaDirect™* 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	AD201-0l	AD201-02
AD1 Buffer	4ml	20ml
AD2 Buffer	1ml	5 ml
AD3 Buffer	4ml	2x10 ml
2X KiaDirect™ PCR Mix (+dye)	1ml	5 ml
ddHp	5 ml	25 ml

Amount of Starting Material

Material	Amount
Mammalian Cells	1-5x10^6 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	30mg
Blood	20 µl

1. Genomic DNA extraction
   Mix 40 µl of AD1 buffer with 10 µl of AD2 buffer. For more samples, premix AD1 buffer with AD2 buffer at ratio of 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 dow
   - Hair Shafts
     Cut hair into pieces, add the mixture of AD1 and AD2, mix thoroughly by pi petting up and dow
   - Animal Tissues
     Cut up tissues with sterile scissors or blade, add the mixture of AD1 and AD2, mix thoroughly by pipetting up and down.

FOR RESEARCH USE ONLY

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- Blood
  Directly add blood into the mixture of AD I and AD2, mix thoroughly by pipetting up and dov

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 40 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 µlM)	0.4 µL	0.2 µlM
Reverse Primer (10 µlM)	0.4 µL	0.2 µlM
2X KiaDirect™ PCR Mix (+dye)	10 µL	1X
DDW	Variable	-
Total volume	20 µL	-

Thermal cycling conditions

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

The extracts can be stored at 4°C for three months or at -20°C for six months.

If non-specific amplification bands are observed, adjust the annealing temperature or properly reduce the quantity of template used.

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 ofPCR cycles (no more than 40 cycles).

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