

EzWay™ Mouse Tail Direct PCR Kit

- 1. Catalog No.** K0568600
- 2. No. of Applications** 1 Set
40 reactions at 50ul PCR Volume / 100 reactions at 20ul PCR volume
- 3. Storage** 1 year at -20°C, 1 month at 4°C
(The product is able to be shipped on blue ice and should be stored immediately at -20°C.)

4. Contents

Component	Cat. No	Volume
2X EzWay™ Direct Hot Taq PCR MasterMix	K0568210	1.0 ml
5X Mouse Tail Direct Lysis Buffer	K0568610	1.5 ml
4X Magic Buffer	K0561031	1.0 ml

5. Description

EzWay™ Mouse Tail Direct PCR Kit enables DNA amplification directly from a mouse tail without DNA extraction. Just cut the mouse tail and incubate it in the lysis buffer provided in the kit for 10 minutes and then use 1ul of lysate for PCR. It doesn't require overnight incubation or Proteinase K (PK) treatment.

- Fast and simple method for genotyping of transgenic mouse without DNA purification
- Just 10 minutes incubation required
- No need to add Proteinase K
- Saving enormous amounts of material and time
- Optimized MasterMix type containing EzWay™ Hot Taq PCR enzyme, dNTP, Direct PCR Buffer, MgCl₂, Red dye and additives
- Direct loading of PCR products without adding red dye

6. Sample Treatment

1. Cut mouse tail with 1-2 mm length (or 5-10 mg), and add lysis buffer. Incubate the tube at 60°C for 10 min in water-bath/rotary hybridization oven or PCR thermal cycler (Vortex at times will be helpful for lysis). DNA molecules from the tail tissue are released.
2. Vortex and spin down briefly.
3. Directly use 1ul supernatant of crude lysate per 50ul PCR reaction.
Note: Mouse Tail Direct Lysis Buffer contains highly concentration EDTA and detergents, so higher amounts of lysate may affect the ability of the buffer to neutralize the inhibitor.

7. PCR Amplification

1. Prepare the Mix according to the table below.

Component	Final Concentration	Volume/reaction	
2X EzWay™ Direct Hot Taq PCR MasterMix	1X	10 µL	25 µL
5' Primer	0.1 - 0.5 uM	Variable	Variable
3' Primer	0.1 - 0.5 uM	Variable	Variable
4X Magic Buffer	0.-25% (v/v)	0-5 µL	0-10 µL
Distilled water	-	Variable	Variable
Template	-	~1 µL	1-2 µL
Total reaction volume		20 µL	50 µL

Note:

1. EzWay™ Direct Hot Taq PCR MasterMix contains 3.0 mM MgCl₂ (final 1.5 mM MgCl₂). Generally, 1.5 mM MgCl₂ may gives satisfactory PCR results, but for higher MgCl₂ than 1.5 mM, add MgCl₂ separately.

2. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 % (v/v) of final PCR volume.

2. Mix gently.

3. When using a thermal cycler without a heated lid, add approximately 100ul of mineral oil on top of the mixture.

4. Perform thermal cycling.

Step		Temp.	Time	Cycles
Initial Denaturation		95°C	5 min	1
Cycling	Denaturation	94°C	0.5-1 min	35-45
	Annealing	50-68°C	0.5-1 min	
	Extension	72°C	1-5 min (~1kb/imin)	
Final Extension		72°C	10 min	1

Note:

- a. Primers should be 15 to 30 bases in length and near 50% G+C content.
- b. Magic Buffer will improve DNA amplification of templates that have a high G+C content and a high degree of secondary structure. We recommend that the volume added should not exceed 25 %(v/v) of final PCR volume.
- c. If chemically modified hot start Taq PCR enzyme is used, the best initial denaturation time is 10-15 minutes at 95°C.

5. The amplified DNA can be detected by various electrophoresis techniques. The most common techniques are agarose or polyacrylamide gel electrophoresis depending on the size of the amplicon.

TAE gel is highly recommended because the bands may be detected dispersed and/or distorted in TBE gel.

8. Related Products

Cat. No.	Product	Size
K0568001	EzWay™ Direct PCR Buffer (5X)	500 ul
K0568011	EzWay™ Direct PCR Buffer, for AmpliTaq Gold	500 ul
K0568002	EzWay™ Direct PCR Buffer Set	1 Set
K0568012	EzWay™ Direct PCR Buffer Set, for AmpliTaq Gold	1 Set
K0568010	EzWay™ Direct Taq Master Mix (2X)	1 ml
K0568020	EzWay™ Direct Taq Master Mix w/o dye (2X)	1 ml
K0568210	EzWay™ Direct Hot Taq PCR MasterMix	1 ml
K0568500	EzWay™ Direct ApoE Genotyping Kit	50 Tests

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