Mouse Direct PCR Kit

Description

The Mouse Direct PCR Kit provides a fast preparation and PCR amplification that is specifically designed for mouse genotyping. The Buffer L and Protease Plus rapidly digest mouse **tail**, **ear** and **toe** to release intact genomic DNA that can be used directly as the template for PCR amplification. By using this kit, the digestion process only takes **15 minutes**. In addition, the 2x M-PCR OPTI Mix (which includes an optimized Taq Polymerase) ensures high amplification efficiency of target DNA.

Components

Contents	Cat#:B40013	Cat#:B40015
Buffer L	20 ml	25 mL x 2
Protease Plus	0.4 mL	1 mL
2 x M-PCR OPTI Mix	1 mL x 2	1 mL x 5

• Buffer L: Lysis buffer
• Protease Plus: For rapid and efficient digestion of mouse tissue in only 15 minutes!
• 2x M-PCR OPTI Mix: Includes Bimake's optimized Taq DNA polymerase, dNTPs, $MgCl_2$, and reaction buffer.

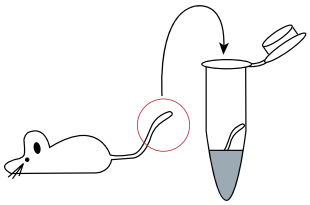
Storage

• 2x M-PCR OPTI Mix and Protease Plus should be stored at -20°C for 2 years. If the PCR Mix is to be used frequently, it can be stored at 4°C for up to 10 days.
• Buffer L should be stored at 4°C.

Notice

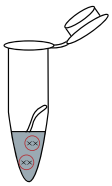
1. Ensure all tools are clean to avoid cross-contamination when collecting samples.
2. All reagents have been optimized for use together and any modifications or alternative uses are prohibited.
3. Before each step, check to make sure every reagent is fully liquid and resuspended prior to use.
4. When performing a digestion, the cut fragment must be completely immersed in the digestion solution.

Protocol



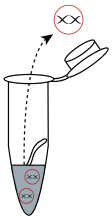
Add protease mixture to the tissue tub

Tissue Digestion



Incubate at 55°C for 15 minutes to release genomic DNA

Protease Inactivation



After digestion, incubate at 95°C for 5 minutes to inactivate the proteases

PCR

After centrifuge, 12,000rpm for 10 min, the supernatant of digested solution can be used as the template for PCR

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Tel: (713)732-2181 Fax: +1-866-747-4781
E-mail: order@bimake.com

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Tel: +49-89-46148500 Fax: +49-89-461485022
E-mail: eu.order@bimake.com

Genomic DNA Preparation

1. Place the mouse tail, ear, or toe in a 1.5 mL centrifuge tube.
2. Thoroughly mix 100 µL of fresh Buffer L with 2 µL of Protease Plus for a single sample in a separate tube.
3. Add the protease mixture to the mouse tissue tubes with the tissue cut end submerged in it, then incubate at 55°C for 15 minutes (incubation times may vary, depending on samples digestion rates).
4. After the digestion process, incubate at 95°C for 5 minutes to inactivate protease. The tissue lysate can now be used as a PCR template.

PCR Genotyping

5. Add ddH_2O primers, template, and 2 x M-PCR OPTI Mix into a PCR tube according to the recommended concentrations. Give the mixture a quick spin in the centrifuge and load into PCR amplifier to begin amplification.

PCR Reaction Components	20 µL Reaction Volume (µL)	50 µL Reaction Volume (µL)
ddH_2O	8	21
Forward Primer (10 µM)	0.5	1
Reverse Primer (10 µM)	0.5	1
Template	1	2
2 x M-PCR OPTI Mix	10	25

Note: The volume of template is Variable.

Temperature (°C)	Time	Cycles
94	5 min	1
94	20 sec	35
50-65	30 sec	
72	X min (2 kb /min)	
72	5 min	1
12	--	1

Trouble Shooting

Please review the following for trouble-shooting options when you encounter technical difficulties. Alternatively, feel free to contact Bimake technical support directly.

Problem	Potential Cause(s)	Suggestion(s)
No amplification product in test or control samples	Amplification reaction was incorrectly set up	Optimize the proper reaction set up
	Improper storage has led to loss of activity of PCR reagents	Replace all components with fresh reagents
	Primers are not optimal and did not anneal	Redesign primers
Amplification worked in the control samples, but not in test samples	Digestion was incomplete	Extend digestion time up to 30 minutes at 55°C
	Lysis solution was mixed with PCR mixture for too long	Collect fresh mouse tail samples for genomic DNA extraction
	The quantity of the amplification product was not sufficient	Increase the number of PCR cycles to 35-40 to yield more amplification product
Non-specific amplification product(s)	Annealing temperature was too low	Increase the annealing temperature
	The number of PCR cycles was too high	Decrease the number of cycles to 30-35
	Primer concentration was too high	Decrease primer concentration
	Template concentration was too high	Dilute template in purified H_2O or TE buffer