# All$^{ele}$-In-One Mouse Tail Direct PCR System

Allele-in-One mouse tail direct PCR buffer releases DNA from mouse tails for genotyping PCR. A one-step reaction using the single buffer system is sufficient for preparing DNA as PCR template; phenol extraction, precipitation, or any further purification is not necessary. The buffer contains a combination of enzyme(s), detergents, and other chemical reagents that will lyse the mouse tail tissues or other tissues without destroying DNA. An aliquot is then directly used as template in a genotyping PCR reaction. It is best used in combination with All$^{ele}$-in-One PCR MasterMix (All$^{ele}$-in-One Mouse Tail Direct PCR Kit, **ABP-RD-2112, 2113**), specifically formulated to accommodate DNA template from lysis buffer that contains proteases.

This buffer can also be used to prepare genomic DNA for direct PCR from other tissues, such as mouse ear, rat tissues, etc. Slight adjustment and optimization may be needed for each type of tissue.

**All$^{ele}$-in-One Mouse Tail Direct PCR** system offers a variety of advatages including:

♦ **Simple buffer system:** Unique from Allele Biotech Enzymes, this single buffer system is the only product that contains everything for lysis, no need to add protease K or any other components before use.
♦ **Single step reaction:** The incubation is to be performed at a convenient 50-55°C temperature for 0.5 hours or longer, and the lysate is ready for PCR.
♦ **Consistent:** Works with extremely high success rates and is fully guaranteed if used in combination with Allele-in-One PCR MasterMix system.
♦ **Lowering costs:** Adds only 40 cents or less to each genotyping sample while saving enormous amounts of material and time from traditional methods.

## Box 1 | Genotyping Results


Mouse tail biopsy at ~0.3cm was incubated in 100µL Lysis Buffer for 2 hours at 55oC. Genomic DNA in 1ml lysate was directly amplified in a 10µL PCR reaction (5mL of 2X PCR Mastermix added) for 29 cycles.

*Each batch of reagents is vigorously tested for consistency and stability.*

All$^{ele}$-in-One Mouse Tail buffer is suitable for many applications such as:
♦ Lysis of mouse tail samples for genotyping PCR

♦ Lysis of other sample types (e.g. mouse ear, yolk sac or tissue culture cells).

The Mouse Tail Direct PCR Kit includes All$^{ele}$-in One PCR MasterMix, which is optimized for DNA template prepared with the lysis buffer but can also be used for most routine PCR reactions.

## Box 2 | Product List

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|---|---|
| **MouseTail Direct PCR Buffer** *100µl per reaction* | |
| ABP-PP-MT01100 | 100 rxns |
| ABP-PP-MT01500 | 500 rxns |
| **Mouse Tail Direct PCR Kit** *10µl per reaction** | |
| ABP-PP-MT02100 | 100 rxns |
| ABP-PP-MT02500 | 500 rxns |

*All Mouse Tail PCR Kits contain **2X PCR Mastermix,** also sold separately:
Cat #: **ABP-PP-MM02961**

**Storage: -20°C and -80°C**

## Protocols

**1.** Prepare 0.3 cm mouse tail biopsy sample.

**2.** Combine 100 µl Lysis Buffer with biopsy sample in 1.5mL reaction tube.

**3.** Incubate at 50-55°C, with or without rotation, for 30 min or overnight.

**4.** For each new PCR primer pair, use 0.05 to 0.5µL of the lysate as PCR template while optimizing the PCR conditions. Once determined, the volume from the tissue lysate will be typically between 0.2 and 0.5µL. This amount should be used in all future reactions with the same primer pair.

**5.** 2X PCR MasterMix should be added as half of the final reaction volume.

♦ *Please see reverse side for Recommended PCR Protocol & Troubleshooting*

**Box 3 | Recommended PCR Setup**

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|---|---|
| **All$^{ele}$-in One PCR 2X MasterMix** | 5µL |
| **Lysate** | 0.05 - 0.5µL** |
| **Forward Primer** | 0.2 - 0.5µL** |
| **Reverse Primer** | 0.2 - 0.5µL** |
| **Fill to 10µL with nuclease-free water** | |

**Lysate and primer concentrations vary based on primer pair.

**Trouble Shooting:**

***1) No PCR products or inconsistent results from time to time:***

Most of the time this is caused by too much genomic DNA. This inhibits the PCR reaction, especially from samples that are prepared by overnight incubation. Try using less lysate as PCR template.

Another explanation may be because of Insufficient lysis. Occasionally the age of the animal or how the tails have been stored determine if it requires a longer incubation at a higher temperature or in a larger volume of lysis buffer (e.g. 200 µl for mice older than 3 months).

***2) Smeared DNA bands:***

This may be caused by too much debris from lysed tissue, which interferes with the Taq polymerase. Add a 3-10 min centrifuge step using a microcentrifuge at top speed and transfer the upper portion of the lysate to PCR reactions.

Also too much time between setting up and starting PCR reactions may cause Taq to degrade. Possible improvements include: Setting PCR contents on ice and moving quickly to a PCR machine or heating the lysate for 15 min at >80°C after the 50-55°C incubation before using as PCR template.