AbTaq Genotyping kit

Catalogue No.	Pack Size
OP-GK-001-100	100 reactions
OP-GK-001-1000	1000 reactions

Product Description

The AbTaq Genotyping kit is designed to minimize handling, time and materials required to perform genotyping. The kit consists of 2 components: (i) TaqLysis Buffer that allows DNA template preparation in a single pipetting step and (ii) a AbTaq 2x Master Mix conveniently formulated to facilitate PCR-based amplification and subsequent direct gel-loading. With less steps needed to prepare and analyse DNA samples, the AbTaq Genotyping kit enables a **simple, fast and cost-efficient** way to amplify DNA fragments from various sources.

Product Contents

Catalog No.	Component Name	Size
OP-GK-001-100	2x AbTaq Master Mix	1 x 1ml
	TaqLysis Buffer	2 x 4ml
OP-GK-001-1000	2x AbTaq Master Mix	10 x 1ml
	TaqLysis Buffer	1 x 80ml

Product Application

While the genotyping protocol described here uses mouse tail as a source of DNA, the same protocol can be applied to any animal tissue.

Component	Features
TaqLysis Buffer	The buffer is formulated to allow DNA sample preparation by simply boiling the tissue. This one-step procedure reduces time, handling and materials that are typically required for purification of genomic DNA.
2x AbTaq Master Mix	The 2x concentrated mix contains all the components needed for a PCR reaction except for the template, primers and water. At the heart of the 2x AbTaq Master Mix is our engineered AbTaq polymerase, which is capable of robust amplification even in suboptimal conditions such as crude / impure DNA sample preparations. The mix is optimised for optimal PCR performance and direct loading of the PCR products on the gel, without the need to add a DNA loading buffer. The all-in-one 2x AbTaq Master Mix thus further reduces time, handling and materials needed for PCR reaction setting set up and analysis.

Product Protocol

The protocol consists of 4 steps. The suggested PCR reaction set-up (Step 2) and PCR amplification conditions (Step 3) can be used as a starting point for further optimization.

Step 1. DNA sample preparation	
1.	Heat a water bath or a heating block to 95°C.
2.	Collect 0.3 – 0.5 mm of mouse tail into a 1.5 mL microcentrifuge tube and add 50 - 80 µL of TaqLysis Buffer to the Eppendorf tube. The tail should be completely immersed in the buffer.
3.	Incubate the microcentrifuge tube at 95 °C for 30 min. *!Important safety consideration: ensure the tube is securely closed.*
4.	Place the microcentrifuge tube into the microcentrifuge and spin for 5 seconds at maximum speed to collect the condensation.

Step 2. Setting up a PCR reaction (Final Volume: 20ul)		
Components	**Final Concentration**	**Volume**
2x AbTaq Master Mix	1x	10 µL
Forward primer (10 µM)	400 nM	0.8 µL
Reverse primer (10 µM)	400 nM	0.8 µL
DNA template	Variable	2 µL
Water	To final vol of 20ul	4.8 µL

Step 3. PCR amplification conditions			
Steps in PCR cycle	**Temp. (°C)**	**Time**	**Cycle**
1: Initial denaturation	95	3 min	1
2: Denaturation	95	15 s	35
3: Annealing	50 - 60	15 - 30 s	
4: Extension	72	30 s / kbp	
5: Final extension	72	5 min	1
6: Hold	10	∞	1

Step 4. Analysis of the PCR products
Load the entire PCR reaction directly onto a 1-2 % agarose gel for electrophoresis.

Important considerations

DNA sample preparation

When using tails from older mice (age > 30 days), a longer incubation time at 95 °C (up to 1 hr) in the Genotyping Lysis buffer might be required. We also recommend mechanically disintegrating the older tail after the lysis by using a 1 mL pipette tip and pressing the tail against the tube wall followed by a quick centrifugation at maximum speed to bring everything to the bottom of the tube. For better stability, we suggest storing the mouse tails and the lysates at -80 °C instead of -20 °C.

A 3 - 5 mm punch of **any tissue** can be used for genotyping with the described protocol. Avoid using larger tissue pieces as it might have an inhibitory effect on the PCR reaction.

PCR reaction

Volume of 20 µL is sufficient for most genotyping assays. The reaction volume might be scaled down or up if necessary. For multiplex PCR, we do not recommend using <20 µL reaction volume.

Primers should be designed according to standard PCR guidelines and are typically used in the concentration range of 100 - 400 nM. A concentration of 400nM / primer is a good starting point for multiplex PCR.

Template From 0.5 to 6 µL of the tail lysate has been successfully used for a PCR amplification. For **problematic templates with high GC content**, up to 10% of DMSO can be added to the PCR reaction to ease the DNA secondary structures.

PCR amplification conditions

Initial denaturation of the DNA strands is performed at 95 °C for 2 - 4 min and for cycling, we recommend DNA **denaturation** to be set at 95 °C for 15 s.

With optimal primer design, a successful PCR amplification can be achieved over wide range of **annealing temperatures**. +/– 2 °C of the lowest primer T_m is usually ideal. If possible, the optimal annealing temperature should be empirically determined by performing a temperature gradient PCR in the range + / - 5 °C of the calculated T_m of the primers. If DMSO is added to the PCR reaction, the annealing temperature should be reduced by 0.75°C for every percentage of DMSO added (by volume).

For genotyping, the amplicon size is typically < 1 kbp and thus 1 min **extension** at 72 °C will be sufficient for most PCR reactions. This crude DNA extraction method is *not* recommended for amplification of PCR products > 1 kbp.

We recommend setting 30 - 40 **cycles** of amplification**.**

The **final extension** step is set to fill any incomplete ends of the newly synthesized strands and is performed at 72 °C for 2 -10 min.

Storage Condition and Stability

The TaqLysis Buffer can be stored at room temperature for at least one year. The 2x AbTaq Master Mix should be stored at -20°C. Avoid repetitive freeze/thaw cycles. If handling smaller sample sizes, aliquoting the 2x AbTaq Master Mix is recommended.

Important Safety Instructions

The TaqLysis buffer contains a strong base thus extra care should be taken when handling the buffer.

Avoid using 1.5 mL microcentrifuge tubes that tend to open when incubated at 95 °C for long period of time. Tube "popping" might result in the splashing of hot liquid into the surroundings. If desired, a weight may be placed on top of the lids to prevent the lids from popping open.

Technical reference and support

Truett G. E. et. al., Vol. 29, No. 1, 2000, BioTechniques.

If you are experiencing difficulties with setting up your PCR reaction and/or PCR amplification conditions, please contact our team with relevant information at **infoab@abasiabiolabs.com**