

Genotyping Kit

Contains:

Product Code	Kit	Lysis Solution	MegaMix-GT
2GTK-100	100 Test Kit	1 x 10ml	2 x 0.5ml
2GTK-250	250 Test Kit	2x 10ml & 1 x 5ml	5 x 0.5ml
2GTK-500	500 Test Kit	5 x 10ml	10 x 0.5ml

Protocol

Lysis:

Perform lysis in a water bath, heating block or thermocycler using the following protocol.

- Thaw Lysis solution and bring to room temperature
- Add mouse ear punch or tail snip tissue (approximately 2-3mm sections) in to 100µl of Lysing Solution
- Vortex the tube for 5 seconds

Incubation:

Step	Temperature	Duration
Lysis	70°C	15 min
Enzyme inactivation	95°C	2 min

- Vortex tube for 5 seconds.
- Spin tube at 2K rpm for 2 minutes to pellet debris
- Use 5µl of clear solution in PCR (20-25µl final volume)
- Store rest of sample at -20°C

PCR:

The Mastermix is a double concentrated PCR Mastermix that contains an inert loading dye. Refreeze the rest of the Mastermix after use. It can be frozen/thawed many times without loss of activity.

Component	Per 20µl Reaction	Final Conc.
Mastermix	10µl	1x
10µM Forward primer	1µl	0.5µM
10µM Revers primer	1µl	0.5µM
Template DNA	As Required	10ng or up to 5µl of crude extract
PCR Grade Water	To make up a final volume of 20µl	N/A

Protocol:

- Thaw the Mastermix
- Add the desired volume in to the PCR tube (as outlined in table above)
- Add DNA, primers and water to an equal volume of the Mastermix
- Overlay with mineral oil if necessary
- Place in a Thermal Cycler

Cycling Profile (Guide Only – user to verify annealing temperature for their specific primers)

Step	Temperature	Time
Initial Denaturation Step	95°C	3mins
Cycle 25-30 times the following		
1: Denaturation	95°C	30 secs
2: Annealing	Optimised annealing temp of primers (generally between 58-68°C)	30 – 60 secs
Elongation	72°C	30 – 60 secs

- After final cycle cool to room temperature