

PR1MA™ 1 Hour Mammalian Genotyping Kit

Description

The 1 Hour Mammalian Genotyping Kit offers fast, efficient extraction and amplification of genomic DNA. Designed primarily for tail snips and ear punches, the kit performs well with a variety of sample types including blood, FFPE tissue sections, buccal swabs, hair and solid animal tissues. The proprietary extraction buffer works quickly, eliminating overnight Proteinase K digestions and produces PCR ready DNA in 10-15 minutes. The included 2X Hot Start Taq Master Mix Red allows for fast PCR of the samples without any clean up or extractions. The red dye in the Master Mix allows PCR products to be loaded directly onto an agarose gel.

- DNA extraction and amplification is as little as 1 hour
- Works with difficult to lyse samples
- Included Taq Master Mix engineered for superior results with crude samples

Storage

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, this product will retain its activity for 12 months from date of receipt. The product may also be stored at 4°C for up to one month.

Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

Quality Control

PR1MA enzymes and reagents are tested under general assay conditions for activity, reproducibility, efficiency, heat activation, sensitivity, and absence of nuclease contamination and nuclease activity. This product is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

MidSci is not responsible for consequential or incidental damages, direct or indirect, resulting from use of this product. MidSci guarantees the performance of this product as described when used in accordance with these instructions.

Extraction Protocol

The extraction buffer will work with a variety of sample types. Below are the amounts/size for common genotyping samples in 100µl of extraction mix.

Sample	For 100µl extraction
Tail Snip	1 to 2mm (2.5 to 6mg)
Ear Punch	2 to 4mm ² (2.5 to 6mg)
Hair follicles	up to 10
Buccal Swab	1 swab use 300µl buffer volume
Animal Tissue	3mg to 30mg Yields may be reduced for samples over 30mg
FFPE Tissue	1mm ³ or 2mm ² of 10µm section
Mammalian Blood	2 to 8µl fresh/EDTA blood 2mm ² FTA, FTA elute or Guthrie cards

1. Add the following to a microcentrifuge tube:

Component	For 100µl reaction
Sample	See table above
5X Buffer A*	20µl
10 Buffer B*	10µl
PCR grade water	70µl

*Buffer A is lysis buffer, Buffer B is protease buffer.

2. Incubate at 75°C for 5 minutes, vortexing 2X during incubation.
3. Deactivate the protease by incubating at 95°C for 10 minutes.
4. Add 900µl of PCR grade water and spin in a high speed microcentrifuge to pellet any debris. Note: There may still be some solid sample after the lysis step. This is normal.
5. Pipette off the lysate and freeze or continue with PCR using the Hot Start Taq Master Mix Red included in the kit.

Technical Support

For trouble-shooting and tech support, contact us at tech@midsci.com or call 800 227-9997.

PCR Reaction setup

Prepare the reaction as follows:

Component	25 µl reaction	50 µl reaction	Final concentration
PR1MA 2X Hot Start Taq Master Mix Red	12.5 µl	25 µl	1X
Forward Primer (10µM)	1.0 µl	2.0 µl	400 nM
Reverse Primer (10µM)	1.0 µl	2.0 µl	400 nM
Supernatant from extraction step	0.5 - 1.0µl	1.0-2.0µl	variable
PCR-grade water	to final reaction volume		

For other volumes, adjust the amount of each component accordingly.

Gently mix the solution. If needed, spin briefly in a microcentrifuge to bring reaction mixture to the bottom of the tube. Transfer samples to a thermal cycler and begin cycling. Be sure to include an initial activation/denaturation step of 1-2 minutes.

Routine PCR Cycling

Step	Temperature	Time
Enzyme activation	95°C	1-2 minutes
	95°C	15 seconds
25-40 cycles	55°C to 67°C*	15 seconds
	72°C	15 seconds per Kb

*Annealing temp determined by user

After PCR, samples can be loaded directly on an agarose gel. Master Mix contains an inert red tracking dye which migrates with the 600bp DNA fragment in 1% gel.

Package contents and reordering

The 1 Hour Mammalian Genotyping Kit is available in 80 and 400 reaction packs. Kit includes lysis buffer, protease buffer and 2X Hot Start Taq Master Mix Red.

1 Hour Mammalian Genotyping Kit, Sample Pack

Catalog number PR1300-MG-5
Includes buffer and Master Mix for 8 reactions.

1 Hour Mammalian Genotyping Kit, 80 Rxns

Catalog number PR1300-MG-80
Includes 1.6ml of 5X Buffer A, 0.8ml of 10X Buffer B and 2ml of 2X Hot Start Taq Master Mix Red in 1.0ml aliquots.

1 Hour Mammalian Genotyping Kit, 400 Rxns

Catalog number PR1300-MG-400
Includes 8ml 5X Buffer A in 1.6ml aliquots, 4ml 10X Buffer B in 0.8ml aliquots, 10ml 2X Hot Start Taq Master Mix Red, in 1.0ml aliquots.

MidSci offers a full line of PCR enzymes and master mixes. Visit www.midsci.com for details.

1 Hour Mammalian Genotyping Kit PR1300-MG

Lysis Buffers and PCR Master Mix
Package contains:
1.6ml of 5X Buffer A, 0.8ml of 10X Buffer B and Hot Start Taq Master Mix Red (2x1.0ml)
80 reactions, Based on 100µl total reaction volume
Store at -20°C upon receipt

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