GenoCheck

Buccal swab genotyping kit for rodents

Storage and shelf-life

Expiry dates are indicated on the labels.

Component	Storage
2 x Hot-Start Master Mix	- 20°C Daily used aliquots might be stored at 4°C-8°C in the dark.
Swabs	room temperature
GenoCheck Lysis buffer	room temperature

Product description and kit contents

The 7Bioscience GenoCheck kit allows non-invasive genotyping of rodents. The kit includes buccal sampling swabs, lysis buffer for the fast and efficient cell lysis in less than 20 min and a sensitive 2x Hot-Start PCR Master Mix. This 2x Hot-Start PCR Master Mix includes a recombinant, thermostable Taq from Thermus aquaticus. The optimized buffer system contains potassium chloride as well as ammonium sulfate and allows the amplification of difficult templates (e.g GC-rich). A red-colored loading dye is included in the Master Mix which allows for direct loading onto agarose gels after PCR.

Swabs	200 pcs
GenoCheck Lysis buffer	20 ml
HotStart PCR Master Mix with loading dye	2 x 1 ml
Manual	1

Reagents and equipment to be supplied by user

- Microcentrifuge
- Thermo shaker (99°C)
- Disposable gloves
- Pipette and pipette tips
- Vortex mixer
- Safe-Lock reaction tubes (1.5 ml or 2.0 ml)
- Micropipettes
- Pipette tips
- PCR reaction tubes (0.1 ml or 0.2 ml)

- Sterile distilled/nuclease-free water
- Primer pairs

Sample collection

Proper sample collection is essential to ensure sample material is sufficient for DNA extraction. As the DNA for genotyping is extracted from the cells of the buccal mucosa, collecting saliva is not sufficient. Furthermore, avoid collecting cells from the tongue's keratinized epithelium.

1. Open the outer packaging of the swab and open the reaction tube.

2. Hold the mouse firmly by fixing the neck skin between thumb and index finger. Pulling on the neck skin will result in opening of the mouth. Insert the brush-like end of the swab into the mouse's mouth. Rotate the swab at least three times around its axis to collect mucosa cells from the inner cheek.

   ***Note**: Proper fixing of the mice might require some practice. Results are usually better sampling only one cheek!*

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3. Place the brush-like end of the swab carrying the buccal cells into the reaction tube and cut off with scissors. The speciman can be used directly for lysis. Alternatively, the sample might be stored at 4°C for up to 24 hours or at -20°C for several days.

Cell lysis and DNA extraction

Cloudy precipitates may form in the buffer, these do not affect the function of the buffer. Shake the buffer well before each use to ensure even homogenisation of buffer components.

1. Shake the buffer well before use.
2. Pre-heat thermo shaker to 99°C.
3. Add 50 -100 µl GenoCheck Lysis Buffer to a reaction tube containing a swab.
4. Resuspend by vortexing for at least 5 sec.
5. Incubate the sample on the pre-heated thermo shaker for 8 min at 99°C while continuously shaking (e.g. 1.400 rpm).
   ***Note**: Ensure that reaction tubes are tightly closed!*
6. Cool down sample tube for 2 min at RT.
7. Centrifuge at max. speed (>10.000 x g) for 2 min and remove swab.
8. Use 1-5 µl for PCR analysis.

Genotyping by PCR

Thaw and mix the 2x Master Mix gently by inverting 8 - 10 times prior to use.

Do not vortex the master mix to prevent damage of the enzyme.

Pipetting Scheme

Components	20 µl Reaction	Final concentration
2 x Hot-Start Master Mix	10 µl	1 x
Forward primer (i.e. 5 pmol/µl)	variable (i.e. 2 µl)	0.1 - 0.4 µM
Reverse primer (i.e. 5 pmol/µl)	variable (i.e. 2 µl)	0.1 - 0.4 µM
Template DNA	1 – 5 µl	0.1 - 20 ng/reaction
Sterile distilled water	adjust to 20 µl final volume	

We recommend to use positive and negative controls in each run.

Standard Amplification Protocol

Step		Time	Temperature
Initial denaturation		1-5 minutes	92 - 95 °C
25 – 50 cycles [1)]			
Denaturation		5 – 10 seconds	92 - 95 °C
3 Step Protocol	Annealing	5 seconds	*60 °C - 68 °C*
	Extension	20 seconds	72 °C
2 Step Protocol	Annealing/Extension	40 seconds per 1kb amplicon length	60 °C – 72 °C

[1)] When working with low copy numbers high number of cycles should be used

Note:
Usually the optimal annealing temperature is 2 °C - 5 °C below the melting temperature of the primers.
For maximum yield and specificity, annealing temperatures, annealing time, extension time and cycle numbers should be optimized for each template and primer pair.

Order details

Product	Catalog #
GenoCheck Kit	7BS-GenoCheck

Components available	Catalog #
Swabs (100 pcs)	NSC-96000
GenoCheck Lysis buffer (20 ml)	7BS-GCLB-20ML
2x Hot-Start PCR Master Mix (1 ml)	7B-57412-1ML
Scissors	7BS-GCS

Note: *Get one free scissors with your first order. Contact us via order@7bioscience.com.*

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