Squirrel Monkey Retrovirus Detection Kit

Contamination Control Kits

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<table>
<thead>
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<tbody>
<tr>
<td>PP-403 S</td>
<td>10 reactions</td>
<td></td>
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<tr>
<td>PP-403 L</td>
<td>50 reactions</td>
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For in vitro use only
Quality guaranteed for 12 months
Store at -20°C
Aliquoting of reagents and handling on ice is recommended

Kit contents

Hot Start Polymerase (red cap)
S pack: 15 µl
L pack: 60 µl

Mastermix 1 (green cap)
S pack: 250 µl
L pack: 1.25 ml

Mastermix 2 (yellow cap)
S pack: 250 µl
L pack: 1.25 ml

Positive Control DNA (white cap)
S pack: 10 µl
L pack: 50 µl

Negative Control DNA (blue cap)
S pack: 10 µl
L pack: 50 µl

Additionally required material
- pipettes and filter tips
- PCR tubes
- micro centrifuge
- PCR thermal cycler
- agarose gel and electrophoresis system

Description
Squirrel Monkey Retrovirus (SMRV) Detection Kit provides a highly sensitive, easy-to-perform and rapid tool for detection of SMRV contaminations in cell cultures or other biological materials. The kit is based on the amplification of 2 conserved coding regions of SMRV by PCR resulting in characteristic fragments of 186 bp and 197 bp. It allows the detection of the provirus with very high sensitivity. Due to this sensitivity, please pay special attention to avoiding cross contaminations.

Protocol

Preparation of fresh cells
Transfer 1 x 10^6 cells to a sterile vial.
- to err on the side of caution extract the DNA prior to PCR with Jena Bioscience genomic DNA Preparation Kit
alternatively use a thermic and osmotic lyse:
- resuspend in 500 µl sterile water
- incubate samples for 15 min at 95°C
- centrifuge for 1 min at 1000 g to sediment cell debris
- transfer supernatant in a new vial and place on ice

Preparation of other biological material
Testing cells from cryocultures requires extraction of DNA prior to PCR

PCR Reaktion
Prepare 2 Premixes of the following components:

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<tbody>
<tr>
<td>Premix 1</td>
<td>Premix 2</td>
<td>per sample</td>
</tr>
<tr>
<td>Mastermix 1</td>
<td>Mastermix 2</td>
<td>22 µl</td>
</tr>
<tr>
<td>Polymerase</td>
<td>Polymerase</td>
<td>0.5 µl</td>
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For each assay pipet 22.5 µl Premix 1 in a PCR vial, Premix 2 in another and add 2.5 µl of prepared
sample each. For preparation of positive/negative controls add 2.5 µl of Positive Control DNA/Negative Control DNA, or sterile water, respectively. Mix and centrifuge briefly. Place the vials in a thermocycler.

Both reactions (with Premix 1 and Premix 2) may be run simultaneously by the same PCR Program.

**PCR program**

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<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Number of Cycles</th>
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<tbody>
<tr>
<td>94°C</td>
<td>3 min</td>
<td>1</td>
</tr>
<tr>
<td>94°C</td>
<td>30 sec</td>
<td></td>
</tr>
<tr>
<td>66°C</td>
<td>30 sec</td>
<td>35</td>
</tr>
<tr>
<td>72°C</td>
<td>60 sec</td>
<td></td>
</tr>
<tr>
<td>72°C</td>
<td>5 min</td>
<td>1</td>
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**Analysis of amplified products**

- add 5 µl gel loading buffer to each vial, centrifuge and mix briefly
- load 12 µl of each assay onto a 2% agarose gel and run gel electrophoresis

**Gel Analysis**

A gel band at 186 bp with Mastermix 1 and a band at 197 bp with Mastermix 2 is the indicator for SMRV contamination of the sample.

**References**

http://de.wikipedia.org/wiki/Squirrel_Monkey_Retrovirus

