



GenePak[®] RNA PCR test

Kit for viral RNA detection by RT-PCR assay

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User manual



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Assignment of the kit

- 1.1. **GenePak® RNA PCR test kits** are designed for the non-quantitative detection of viral RNA in biosamples (plasma, serum, whole blood, swabs, epithelium cells etc.) by Reverse Transcription – Polymerasr Chain Reaction
- 1.2. Time for the analysis is close to 5 hours.
- 1.3. The kit is designed for 100 reactions including 10 positive and 10 negative control reactions.

2. Basic principles of detection

- 2.1. The kit is based on 2 basic techniques – Reverse Transcription and Polymerase Chain Reaction (PCR).
- 2.2. The kits contain tubes with lyophilized MasterMixes Ready-To-Use for Reverse-Transcription –PCR starting from the purified RNA.
- 2.3. The kits contain random primers for Reverse Transcription initiation, M-MuLV Reverse Transcriptase, Placental Ribonuclease Inhibitor, Taq DNA polymerase and specific primers for PCR. The cloned fragments of viral cDNA with the conservative sequences are used as positive controls.
- 2.4. PCR fragments detection should be done by UV-light after agarose gel electrophoresis with the EtdBr. The fragment of DNA with the specific size indicates the presence of the corresponding RNA template in the biosample.

3. Analytical characteristics

- 3.1. Usage of positive control as a biosample in the test should result in the appearance of the band with the specific size after gel-electrophoresis (Table 2).
- 3.2. Usage of negative control as a biosample in the test should not result in the appearance of the band with the specific size after gel-electrophoresis.

4. The kit characteristics

4.1. The kit contains:

- **(+) control (positive control)**, ready for use, red tubes with lyophilized red powder, 10 tubes;
- **(-) control (negative control)**, ready for use, blue tubes with lyophilized red powder, 10 tubes;
- **RT MasterMix**, ready for use – colorless tubes with lyophilized red powder for the analysis of biosamples, 80 tubes;
- **PCR MasterMix**, ready for use – colorless tubes with blue lyophilized powder for the analysis of biosamples, 100 tubes;
- **RT Diluent**, 1 tube 0,5ml;
- **RT Stop Solution**, 1 tube, 1,0 ml;
- **PCR Diluent**, 1 tube, 1,0 ml;
- **PCR Oil**, 1 tube, 2,0 ml

5. Reverse Transcription

- 5.1. Prepare the relevant amount of colorless tubes with RT MasterMix (red content) for biosamples analysis, one red tube (+) control with red content and one blue tube (-) control with red content
- 5.2. Mark the tubes correspondingly.
- 5.3. Add 5 microliters of RT Diluent in each tube including (+) and (-) controls.
- 5.4. Add in each colorless tubes with RT MasterMix 5 microliters of previously purified RNA.

* Attention!

1. To avoid cross-contamination the following order of samples introduction should be used:

(-) control as the first tube; samples, (+) control as the last tube.

2. Use only filter tips for pipetting samples.

- 5.5. Add 5 microliters of distilled water in (-) and (+) control tubes.
 5.6. Dissolve completely the red content in all tubes. The complete recovery can take up to 10 minutes.
5.7. Incubate tubes 40 min at 50 °C. The content of the tubes should be mixed shortly several times during incubation.
5.8. Add 10 microliters of RT Stop Solution. Incubate 10 min 95 °C.
 5.9. Centrifuge shortly. The samples are ready for PCR.

6. PCR.

- 6.1. Prepare the relevant amount of colorless tubes of PCR Master Mix with blue content for the biosamples analysis, for (+) and (-) controls. Mark them correspondingly..
 6.2 Add 10 microliters of PCR diluent in each tube.
 6.3. Transfer 10 microliters of red colored samples after Reverse Transcription into the corresponding tubes.
 6.4. Add 20 microliter of Mineral Oil if the cycler has no hot lid.
 6.5. Transfer the tubes into the cycler and start the program according to the table below (table 1):

Table 1

N of the programm	Cycle with block temperature	Cycle with active temperature control
	Time, temperature	Time, temperature
1	2 min 95 °C – 1 step	2 min 95 °C 1 step
2	60 sec 95 °C 40 sec 58 °C 60 sec 74 °C 45 cycles	20 sec 95 °C 20 sec 58 °C 40 sec 74 °C 45 cycles
3	120 sec 74 °C 1 step	120 sec 74 °C 1 step

- 6.6. Transfer the tubes into the separate room for electrophoresis.

- 6.7. Load 10 microliters of each sample to agarose gel electrophoresis.
 6.8. Check the gel after electrophoresis under UV light.

7. Results interpretation

- 7.1. The sample from (+) control should give the distinct band with the corresponding size on electrophoresis (Table 2).
 7.2. The sample from (-) control should not give any band on electrophoresis.
 7.3. Occurrence of the band with the corresponding size after analysis of the certain sample indicate the present of the virus RNA in the original biosample.
 7.4. Occurrence of the specific band in the negative control indicates the invalidity of all results due to the contamination.
 7.5. Weak bands above and below the specific bands can be considered as unspecific bands and these bands should not be taken into account.

8. Transportation and Storage

- 8.1. All kits components can be transported at temperature -20 - +30°C.
 8.2. All kits should be store at +2 - + 8°C at least for 6 months.

Table 2.

Virus to be detected	Index	Annealing t°	PCR product size (bp)
<i>Hepatitis C virus</i>	HCV	58 °C	242
<i>Hepatitis C virus-50</i>	HCV-50	58 °C	242, 272
<i>Hepatitis C virus, 1a type</i>	HCV 1a	58 °C	214
<i>Hepatitis C virus, 1b type</i>	HCV 1b	58 °C	240
<i>Hepatitis C virus, 2a type</i>	HCV 2a	58 °C	196
<i>Hepatitis C virus, 2b type</i>	HCV 2b	58 °C	343
<i>Hepatitis C virus, 3a type</i>	HCV 3a	58 °C	238
<i>Hepatitis G virus</i>	HGV	58 °C	208
<i>Hepatitis D virus</i>	HDV	58 °C	417
<i>Hepatitis A virus</i>	HAV	58 °C	365
<i>Hepatitis E virus</i>	HEV	58 °C	215
<i>Enterovirus</i>	EVs	58 °C	175
<i>Coxsackievirus B3</i>	CVB3	58 °C	225
<i>Rubella virus</i>	RuV	58 °C	315
<i>Tick-borne Encephalitis virus</i>	TBEV	58 °C	171
<i>Human Immunodeficiency virus-50</i>	HIV I -50	62 °C	156,169
<i>Rotavirus</i>	HRV	58 °C	266
<i>Norwalk-like virus</i>	NWL	58 °C	399
<i>West Nile Virus</i>	WNV	58 °C	350
<i>Severe acute respiratory synd. v</i>	SARS-CoV-50	58 °C	280, 351
<i>Influenza virus A, general</i>	InfV Agen	58 °C	354
<i>Influenza virus B, general</i>	InfV Bgen	58 °C	196
<i>Influenza virus A-H5</i>	InfV A-H5	60 °C	311
<i>Influenza virus A-H5N1-50</i>	InfV-AH5N1-50	60 °C	311, 339
<i>Human Parainfluenza virus 1</i>	HPIV 1	58 °C	309
<i>Human Parainfluenza virus 2</i>	HPIV 2	58 °C	309
<i>Human Parainfluenza virus 3</i>	HPIV 3	58 °C	309
<i>Human Parainfluenza virus, gen</i>	HPIVgen	58 °C	309
<i>Human Respiratory Syncytial virus</i>	HRSV	58 °C	369
<i>Human Metapneumovirus</i>	HMPV	58 °C	228