

PseeR HCV Detection PCR kit (100 tests)

The Hepatitis C virus (HCV) is a single-stranded positive-sense RNA virus, its genome contains ~ 9600 nucleotides. HCV can be transmitted by blood and blood products to humans. The *PseeR* HCV detection kit detects and quantifies HCV in plasma and serum samples. The principle of detection of the assay kit is based on two steps, first is the reverse transcription step where HCV genomic RNA is reversely transcribed into cDNA, and the second step is the target amplification and fluorescent detection that are monitored on real-time PCR instruments.

***PseeR* HCV detection kit features**

- M-MLV Reverse Transcriptase enzyme lacking RNase H activity, so it has high thermostability.
- Hotstart high-fidelity Taq polymerase to ensure high specificity and avoid non-specific amplifications.
- High sensitivity, detection limit as low as 100 I μ /mL.
- Detection of all HCV genotypes (1-6).

Kit components

components	Quantity	Volume
<i>PseeR</i> HCV Buffer	1 vial	510 μ L
<i>PseeR</i> Enzyme Mix	1 vial	51 μ L
100mM MgSO ₄	1 vial	100 μ L
<i>PseeR</i> primers/probes Mix	1 vial	130 μ L
IC Template	1 vial	500 μ L
Standard 1 (1 \times 10 ⁷ IU/ μ L)	1 vial	50 μ L
Standard 2 (1 \times 10 ⁶ IU/ μ L)	1 vial	50 μ L
Standard 3 (1 \times 10 ⁵ IU/ μ L)	1 vial	50 μ L
Standard 4 (1 \times 10 ⁴ IU/ μ L)	1 vial	50 μ L

PCR Reaction Setup


Component	Volume for 1 Reaction
<i>PseeR</i> HCV Buffer	5 μ L
<i>PseeR</i> Enzyme Mix	0.5 μ L
100mM MgSO ₄	0.5 μ L
<i>PseeR</i> primers/probes Mix	1.5 μ L
IC Template	1.5 μ L

DNA Template/ Standard	16µl
Total volume	25µL

- Internal Control (IC) template can be added to the sample before the extraction to control the extraction and PCR altogether or can be added as a control for PCR only, as follows:

Component	Volume to add to extraction	Volume to add to PCR
IC Template	5µL	1.5µL

Program Setup on real-time PCR instrument

Step	Cycles Number	Program	Analysis mode
Reverse transcription	1	45°C for 20 min	None
Hotstart Activation	1	95°C for 4 min	None
Amplification	40	95°C for 10 sec	None
		60°C for 30 sec 	FAM for HCV & HEX for IC

Results

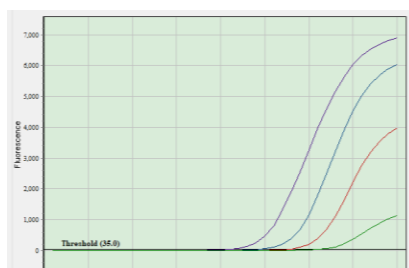


Fig1. HCV results in FAM channel

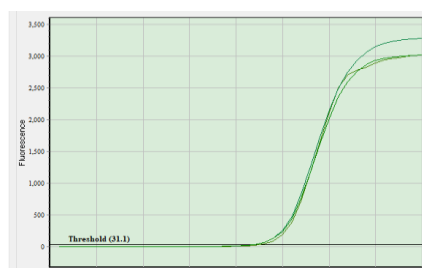


Fig2. IC results in HEX channel

Analysis

Analysis interpretation can be done following the below table:

FAM channel	HEX channel	interpretation
√	√	Positive
-	√	Negative

√	-	Invalid
-	-	Invalid

Compatible instruments

DT-Prime 96, DT-lite 48 (DNA-technology, Russia)

Step-one, Step-one plus, 7000, 7500 (Applied-Biosystems)

Storage and stability

- The *PseeR* HCV Detection PCR kit should be stored in -20°C.
- Repeated thawing and freezing (> 2 x) of the *PseeR* HCV Buffer should be avoided. If it is to be used only intermittently, *PseeR* HCV Buffer should be frozen in aliquots.