

KIT FOR THE QUANTITATIVE DETERMINATION OF HCV RNA

QUANT-C gene

Cat. n. 1.108

The Hepatitis C virus (HCV) is a RNA virus and belongs to the Flaviviridae family. The virus has a single-stranded RNA genome of about 9400bp. This genome consists of a untranslated 5' region highly conserved of about 320-340 bp, a region encoding the poliprotein and at last an untranslated 3' region of about 27-55 nucleotides that contains the poly-A sequence.

The use of the Polymerase Chain Reaction (PCR) allows the detection of viral RNA in biological samples ,as serum, plasma, biopsy. The procedure consists of: - extraction of RNA from samples of serum or plasma; - retrotranscription of viral RNA in cDNA followed by an amplification ,using primers derived by the highly conserved region 5' UTR , of a fragment of 242 bp.

The level of detected HCV-RNA may affect the course of the disease, the response to the pharmacological treatment (with interferon) and it is correlated with the infectivity.

The simultaneous amplification of the samples of RNA and of RNA standard allows to quantify the viral RNA.

Principle of Assay: A)lysis of viral capsid, B)alcoholic precipitation of RNA, C)retrotranscription D)First amplification I, E) Second amplification, F) detection on agarose gel.

Applicability: On extracted and purified RNA.

Tests: 40

REAGENTS AND STORAGE

RETROTRANSCRIPTION and AMPLIFICATION	
Mix RT HCV	-20°C
Reverse Transcriptase (5U/μl)	-20°C
Rnase inhibitor (40U/μl)	-20°C
Mix PCR HCV	-20°C
H ₂ O-DEPC	-20°C
Taq Polymerase (5U/μl)	-20°C

Stability: over 18 months if correctly stored.

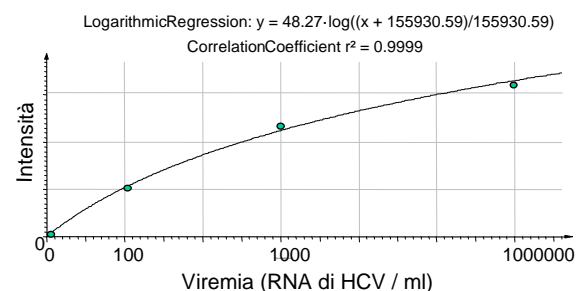
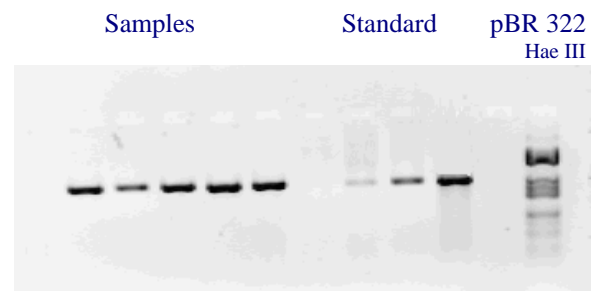
References:

Biochemistry **18**: 5295-5298 (1979)

Science **231**: 379-381 (1986)

Analytical Biochemistry **162**: 156-159 (1987)

ANALYSIS OF RESULTS



The amplification yield of positive RNA-HCV samples is of 242bp. You can quantify the PCR products using a standard curve generated by a known titre standard.