|||||||

A. RNA Extraction

(You can use SinaClon Viral Extraction Kit (DNA & RNA Virus Extraction, Cat No.: EX6065))

- 1. Add $50\mu l^*$ Serum or Plasma to $450\mu l$ cold RNX solution (Cat. No.: EX6101). Vortex the sample to dissolve the clumps. Incubate for 10min on ice.
- 2. Add 100 μl of <code>Chloroform</code>, vortex (3-5sec.) and centrifuge it at 12,000rpm for 5min.
- 3. Transfer the aqueous phase to new tube and add equal volume of <u>Isopropanol</u> (250-300µl). Invert the tube 10 times and then incubate at -20°C for at least 20min. Then Centrifuge at 12,000rpm for 15min.
- Discard aqueous phase and add to the pellet 200µl 70% Ethanol and invert 10 times, centrifuge it at 12,000rpm for 5min.
- 6. Discard aqueous phase and incompletely dry the pellet (RNA) for 20-30min at room temperature.
- 7. Dissolve RNA in $30\mu I$ <u>DEPC treated water</u>, then follow the cDNA synthesis protocol within 3 hours of specimen preparation or store the processed specimens frozen at $-70^{\circ}C$ or colder for up to one month with no more than one freeze-thaw.
- * More sample volume can be applied, add 100µl serum or plasma and then increase components of steps one and two accordingly. During final step, RNA should be dissolved in 30µl of DEPC treated water.

B. Single tube cDNA Synthesis and first PCR Round

Label PCR tubes for cDNA synthesis & first PCR, for test, positive and negative control.

1. Add the following to each tube on ice (Mix & spin before use):

HCV PCR Mix I	33.7µl
RT Enzyme	1µl
Taq DNA Polymerase	0.3µl
Mineral oil	40µl

- 2. Mix the mixture thoroughly by shaking and spin.
- 3. Close reaction tubes or place tray and reaction tubes in a resealable plastic bag and seal the bag securely, do not close reaction tubes at this time. Transfer tubes to RNA extraction site.
- 4. Place RNA tube at 95°C, 1min. and then place on ice.
- 5. Add 10 μ l RNA to each patient tube and 10 μ l Positive control to positive control tube and DEPC-Water to negative control tube. (The final volume of each reaction will be 45 μ l)
- 6. Close tubes, spin the mixture on microfuge 3-5sec and transfer the tubes to preheated thermocycler and start the program:

Cycling parameters		
42°C - 20 min 93°C - 2 min 1 cycle	Then	93°C - 30 Sec 55°C - 40 Sec 72°C - 30 Sec 20 cycles

Cycling parameters may need to be setup with some Thermocyclers.

C. Second PCR Round

1. Add the following to PCR new reaction tube:

HCV PCR Mix II	21.8µl
Taq DNA Polymerase	0.2µl
Mineral oil	20µl

- 2. Close reaction tubes or place tray and reaction tubes in a resealable plastic bag and seal the bag securely, do not close reaction tubes at this time. Transfer tubes to RNA extraction site.
- 3. Add PCR product from first round 3µl. (The final volume of each reaction tube will be 25μ l)
- 4. Transfer the tubes to preheated thermocycler and start the following program:

(3)

Cycling parameters		
93°C – 60 sec 1 cycle	Then	93°C - 30 Sec 55°C - 35 Sec 72°C - 30 Sec 30 cycles

D. Result Analysis

Analyze amplified fragments by loading of 10μ I PCR product in 2% agarose gel directly without adding loading buffer. The presence of 234bp fragments indicates positive test. In smear result without specific fragment (234bp), repeat test with 1/2 dilution of RNA.

Signs

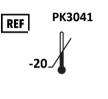
Signs	Definitions	
-20	Temperature range on product use	
	Product end of life detection information	
RUO	For Research Use Only	
	Name and address of the manufacturer of the product	
REF	Product technical code	
Ŷ	Product shipping conditions	

Unit 9, Rouyesh building, Science and Technology Park, Tarbiat Modares University, Pajouhesh Blvd, Tehran, Iran

- S +982191082111
- hi@sinaclon.com
- (www.sinaclon.com



STRP™ Hepatitis C Virus Detection Kit





Components

Contents	Amounts
RNX [™] -Plus	25ml
HCV PCR Mix I	1700µl
HCV PCR Mix II	1100µl
Positive DNA Control	100µl
DEPC Treated Water	2×1ml
RT Enzyme	50µl
Taq DNA Polymerase	25µl
Mineral Oil	3ml

Description

This kit is designed for qualitative detection of HCV RNA in the serum and plasma of Human blood by the method of Single tube RT reaction, followed by nested PCR.

The Reagents Needed:

Chloroform
Isopropanol
70% Ethanol