A. RNA Extraction

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

1. Add 50µl* Serum or Plasma to 450µl cold **RNX** solution (Cat. No.: EX6101). Vortex the sample to dissolve the clumps. Incubate for 10min on ice.
2. Add 100µl of **Chloroform**, vortex (3-5sec.) and centrifuge it at 12,000rpm for 5min.
3. Transfer the aqueous phase to new tube and add equal volume of **Isopropanol** (250-300µl). Invert the tube 10 times and then incubate at -20°C for at least 20min. Then Centrifuge at 12,000rpm for 15min.
5. 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 30µl **DEPC treated water**, then follow the cDNA synthesis protocol within 3 hours of specimen preparation or store the processed specimens frozen at -70°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 10µl RNA to each patient tube and 10µl Positive control to positive control tube and DEPC-Water to negative control tube. (The final volume of each reaction will be 45µl)
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	Then	93°C - 30 Sec 55°C - 40 Sec 72°C - 30 Sec
1 cycle		**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 25µl)
4. Transfer the tubes to preheated thermocycler and start the following program:

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 10µl 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
	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
	Product shipping conditions

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SinaClon BioScience شرکت سیناکلون

STRP™ Hepatitis C Virus Detection Kit

REF **PK3041**

50 TESTS

Wet Ice

RUO

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