STRP™ Hepatitis C Virus Detection Kit

For Research Use Only

Cat. No.: PK3041 **Quantity: 50 Reactions**

Storage: -20°C **Shipment: Wet Ice**

This kit is destined 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.

Kit Contents:

1. RNX™-Plus	25ml	5.RT Enzyme	50µl
2.Mix I	1700 µl	6.Taq DNA Poly.	25µl
3. Mix II	1100 µl	7.DNA Pos.	100µl
4. DEPC-Water	2×1ml	8.Mineral oil	3ml

The Reagent Needed:

1. Chloroform 2.Isopropanol 3.70% Ethanol

RNA Extraction:

Performed in Pre-Amplification 1, Specimen & Control Area

1.Add 50µl* Serum or Plasma to 450µl cold **RNX™-Plus** (#EX6101)solution. Vortex the sample to dissolve the clamps. 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.

4.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 dissolve in 30 µl of DEPC treated water.

Single Tube cDNA Synthesis first PCR Round:

Perform in Pre-Amplification 2, Reagent Preparation Area

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

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

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 resalable plastic bag and seal the bag securely, do not close reaction tubes at this time. Transfer tubes to pre-amplification 1 Area.

4. Place RNA tube at 95°C, 1min and then place on ice.

5. Add **10µl** RNA to each patient tube and **10µl** of positive control to pos. tube and DEPC water to neg. 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	
93°C	30sec	
55°C	40sec	**33cycles**
72°C	30sec	

Second PCR Round

In Pre-Amplification 2, Reagent Preparation Area

1. Add the following to PCR new reaction tube:

1 X 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 resalable plastic bag and seal the bag securely, do not close reaction tubes at this time .Transfer tubes to pre-amplification 1 Area.

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 program:

Cycling parameters:

93°C	60sec	
93°C	30sec	
55°C	35sec	**30cycles**
72°C	30sec	

Result Analysis

Perform in Post-Amplification Area

Analyze amplified fragments by loading of **10µl** PCR products in **2%**agarose gel directly without adding loading buffer.

The presence of **234bp** fragments indicates positive test.

In case smear is observed, test should repeat with ½dilution of RNA sample. For gel electrophoresis use of 100bp Ladder (SL7031) is recommended.

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