STRP™ HIV-1 Detection Kit

For Research Use Only

Cat. No.: PK3121 **Storage: -20°C**
Shipment: Wet Ice **Quantity: 20 Reactions**

This kit is destined for qualitative detection of HIV-1 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	9 ml	**5.** RT Enzyme	20 µl
2. Mix I	780 µl	**6.** Taq DNA Poly.	10 µl
3. Mix II	440 µl	**7.** DNA Pos.	25 µl
4. DEPC-Water	600 µl	**8.** Mineral oil	2 ml

The Reagents Needed :

1. Chloroform **2.** Isopropanol **3.** 70% Ethanol

RNA Extraction

Perform in Pre-Amplification 1, Specimen & Control Area.

1. Add 50 µl Serum or Plasma to 450 µl cold **RNX™-Plus** solution. Vortex the sample to dissolve the clamps. Incubate for 10 min on ice.

2. Add 100 µl of **Chloroform** , vortex (3-5 sec.)and centrifuge at 12000 g for 5 min.

3. Transfer the upper phase to new tube and add equal volume of **Isopropanol** (250-300 µl). Invert the tube 10 times and then incubate at -20°C for at least 20 min.

4. Centrifuge at 12000 g for 15 min.

5. Discard aqueous phase and add to the pellet 200 µl **70% Ethanol** and invert 10 times, centrifuge at 12000 g for 5 min.

6. Discard aqueous phase and incompletely dry the pellet (RNA)for 20-30 min. at room temperature.

7. Dissolve RNA in 30 µl 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 .

Single tube cDNA Synthesis and 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) :

1X PCR MixI	39µl
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 Pre- Amplification 1 Area .
4. Place RNA tube at 95°C, 1 min . and then place on ice .
5. Add 5 µl RNA to each patient tube and Positive control to pos. tube and DEPC-Water to neg. tube.

(The final volume of each reaction will be 45 µl)

6. Close tubes, spin the mixture on microfuge 3-5 sec. and

transfer the tubes to preheated thermocycler and start the program :

Cycling parameters:

42°C- 20 min		93°C-40 Sec
94°C - 2 min	Then	62°C- 40 Sec
62°C - 40 sec		72°C -40 Sec
72°C - 40 sec		
1 cycle		**20 cycles**

Cycling parameters may need to be setup with some Thermocyclers.

Second PCR Round

In Pre-Amplification 2, Reagent Preparation Area:

1. Add the following to PCR new reaction tube:

1X PCR Mix II	22 µl
Taq DNA Polymerase	0.2 µl
Mineral oil	20 µl

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 Pre- Amplification 1 Area .

3. Add PCR product from first round 3 µl.
(The final volume of each reaction tube will be 25 µl)

4. Transfer the tubes to preheated thermocycler and start the program :

Cycling parameters :

93°C - 40 sec
62°C - 40 sec
72°C - 40 sec
35 cycles

Result Analysis

Performed in Post-Amplification Area .

Analyze amplified fragments by loading of 10 µl PCR product in 2% agarose gel directly without adding loading buffer . The presence of **174 bp** fragments indicates positive test.In smear result with out specific fragment (174 bp), repeat the step B,C&D with 1/10 dilution of RNA(eg.10 µl of RNA in 100 µl of DEPC-water).

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