STRP™ HIV-1 Detection Kit
(Short Protocol)

Cat. No.: PR8249C
Shipment: Wet Ice

Storage: -20°C
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. CinnaGen HIV-1, RT-PCR detection kit may be used in clinical medicine to detect HIV-1 RNA.

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

A) 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 .

B) 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 Mix I	39 µl
RT Enzyme	1 µl
Taq DNA Polymerase	0.3 µl
Mineral oil	40 µl

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. If so, please contact CinnaGen's Molecular Biology department.

C) 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

D) 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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