STRP™ HIV-1 Detection Kit

REF PK3121 | Σ 20 TESTS | Wet Ice | RUO | -20

Components

Contents	Amounts
HIV PCR Mix I	240µl
HIV PCR Mix II	290µl
Positive DNA Control	25µl
DEPC Treated Water	1ml

Description

This kit is designed 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.

A. RNA Extraction

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

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 12µl One Step PCR Mix I for each tube on ice (Mix & spin before use):
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. Place RNA tube at 95˚C, 1 min. and then place on ice.
4. Add 5-8 µl RNA to each patient tube and 4µl Positive control to pos. tube and DEPC-Water to neg. tube. (The final volume of each reaction will be 20µl)
5. Close tubes, spin the mixture on microfuge 3-5 sec. and transfer the tubes to preheated thermocycler and start the program:

Cycling parameters		
50°C- 20 min 94°C - 2 min 62°C - 40 sec 72°C - 40 sec **1 cycle**	Than	93°C-40 Sec 62°C- 40 Sec 72°C -40 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:

HIV PCR Mix II	**14.5 µl**
DNase free Water/DEPC Water	**5.5 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 5 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

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 without specific fragment (174 bp), repeat the step B, C & D with 1/10 dilution of RNA (e.g. 10µl of RNA in 100µl of DEPC Treated water).

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
	Product shipping conditions

شکایات مشتری

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