RECOMBINANT HUMAN IMMUNODEFICIENCY VIRUS-1 (HIV-1) p24 ANTIGEN

The Human Immunodeficiency Virus (HIV) viral capsid protein p24 plays important roles in HIV pathogenesis. The p24 antigen (Ag) is the most abundant HIV-1 protein and is a major structural protein within the capsid.[1] It is involved in maintaining the structural integrity of the virus and facilitating various stages of the viral life cycle. This p24 antigen may be detectable within 1–3 weeks post HIV infection and can serve as an early-detection marker compared to anti-HIV antibodies (Abs), which may only be detectable beyond 4 weeks after infection.[2]

The combined detection of HIV antigens (such as p24) and anti-HIV antibodies using the 4th or 5th generation screening assays further helps in the early diagnosis of HIV infection with high accuracy in assays such as ECLIA, ELISA/ELFA and LFA (rapid tests). Hence p24 antigen detection assays play a pivotal role in the early-stage HIV detection and treatment.[3]

Resolve's Recombinant HIV-1 p24 antigen is a 25.4 kDa protein fused to His-tag at N-terminus (207 aa, His-tag), expressed in E. coli and purified by affinity chromatography. It is quality tested through third-party labs and can serve as a control/calibrator for clinical diagnostic applications such 4th and 5th generation Ag/Abs combination tests.

Technical Specifications

A. Purity:

1. SDS-PAGE

Resolve's Recombinant Human Immunodeficiency Virus-1 (HIV-1) p24 antigen is >95% pure as determined by SDS-PAGE (Figure 1).

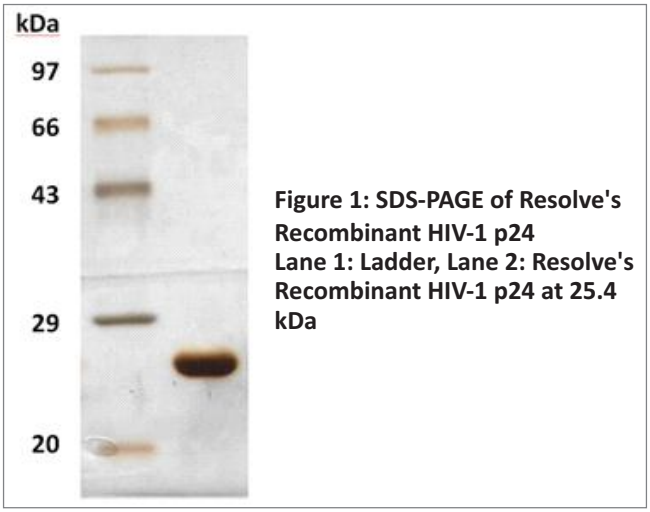

Figure 1: SDS-PAGE of Resolve's Recombinant HIV-1 p24
Lane 1: Ladder, Lane 2: Resolve's Recombinant HIV-1 p24 at 25.4 kDa

B. Detection of Antigenic activity:

1. Method 1: Electrochemiluminescence Assay (ECLIA)

Antigenic activity of Resolve's Recombinant HIV-1 p24 was tested through a third-party diagnostic laboratory —Manipal TRUtest—and significant antigenic activity was detected (Figure 2) on an Electrochemiluminescence Assay (ECLIA) conducted on the Roche Cobas [Elecsys HIV DUO] platform. The HIV DUO platform calculates results automatically based on signal to cutoff ratios (cutoff index, COI) from HIV Ag and anti-HIV Abs.

A.

LABORATORY TEST REPORT

manipal TRUtest
FAST. ACCURATE. RELIABLE

ID	: 177002	Collection	: 14/02/2024, 05:31 PM	Client Name	: Chirag Bidwai(Manipal Trutest)-TH1565
Name	: RESOLVE DIAGNOSTIC A	Received	: 14/02/2024, 05:31 PM		
DOB/Age	: 0 days	Reported	: 15/02/2024, 02:31 PM	Client Address	: Thane
Gender	: Other	Ref. Doctor	: -		

2224021539
ICMR ID : -

Test Description	Value(s)	Unit(s)	Reference Range
HIV-DUO p24 Antigen & Antibody to HIV1 &2 (Serum)			
HIV - DUO Test (Serum, ECLIA)	**Reactive (20.76)**		Non Reactive: < 0.90 Borderline: ‡ 0.90 to < 1.0 Reactive: ‡ 1.0

B.

LABORATORY TEST REPORT

manipal TRUtest
FAST. ACCURATE. RELIABLE

ID	: 177003	Collection	: 14/02/2024, 05:31 PM	Client Name	: Chirag Bidwai(Manipal Trutest)-TH1565
Name	: RESOLVE DIAGNOSTIC B	Received	: 14/02/2024, 05:32 PM		
DOB/Age	: 0 days	Reported	: 15/02/2024, 02:31 PM	Client Address	: Thane
Gender	: Other	Ref. Doctor	: -		

2224021540
ICMR ID : -

Test Description	Value(s)	Unit(s)	Reference Range
HIV-DUO p24 Antigen & Antibody to HIV1 &2 (Serum)			
HIV - DUO Test (Serum, ECLIA)	**Reactive (2.63)**		Non Reactive: < 0.90 Borderline: ‡ 0.90 to < 1.0 Reactive: ‡ 1.0

Figure 2: Reports from Manipal TRUtest for Resolve's Recombinant HIV-1 p24 samples spiked in FBS and diluted to (A) 200 pg/mL (20 IU/mL): COI 20.76; and (B) 20 pg/mL (2 IU/mL): COI 2.63

Note[4]: 1.0 IU/mL of HIV-1 p24 is estimated as 10.0 pg/mL

2. Method 2: Lateral Flow Assay (LFA)

Resolve's Recombinant HIV-1 p24 antigen was also tested on a lateral flow assay—TRUSTline HIV-1 Ab/Ag 4th Gen Rapid Test (Athanese-DX). The test showed clear bands at higher concentrations (1000 ng/mL, 10 ng/mL, 1 ng/mL; Figure 3B/C/D) and a faint band at 625 pg/mL (Analytical sensitivity of the test: 62.5 IU/mL = 625 pg/mL).

Note[5]: The TRUSTline HIV-1 Ab/Ag 4th Gen Rapid Test has a Limit of Detection of 62.5 IU/mL (625 pg/mL) for HIV-1 p24. Resolve's Recombinant HIV-1 p24 antigen was also loaded at 625 pg/mL and a faint band (visible to the naked eye) was observed in the Test line.

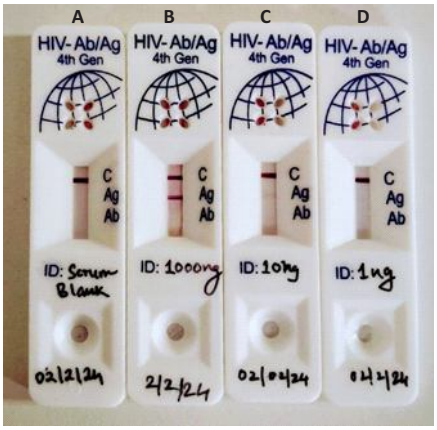

Figure 3: Results of Lateral Flow Assay (TRUSTline HIV-1 Ab/Ag 4th Gen Rapid Test) (A) Blank (FBS): Control line positive but no band at Test line to rule out false positives; (B/C/D) Test: Visible bands at Control and Test lines with Resolve's Recombinant HIV-1 p24 antigen spiked into FBS (B: 1000 ng/mL; C: 10 ng/mL; D: 1 ng/mL).

Resolve's Recombinant HIV-1 p24 antigen exhibited significant antigenic activity as detected with ECLIA method by a third-party laboratory (Manipal TRUtest) and also demonstrated positive results on a Lateral Flow Assay (TRUSTline HIV-1 Ab/Ag 4th Gen Rapid Test) up to the Limit of Detection of the test (625 pg/mL), further validating its utility as a control/calibrator and in innovative point-of-care diagnostic devices.

References

[1]Anderson AM, Tyor WR, Mulligan MJ et al SL. Measurement of Human Immunodeficiency Virus p24 Antigen in Human Cerebrospinal Fluid With Digital Enzyme-Linked Immunosorbent Assay and Association With Decreased Neuropsychological Performance Clin Infect Dis. 2018 Jul 1; 67(1): 137–140. doi: 10.1093/cid/ciy056

[2]Bystryak S, Acharya C. Detection of HIV-1 p24 antigen in patients with varying degrees of viremia using an ELISA with a photochemical signal amplification system. Clin Chim Acta. 2016;456:128-136. doi:10.1016/j.cca.2016.02.022

[3]Branson, Bernard M. et al. (2014). Laboratory testing for the diagnosis of HIV infection: updated recommendations. Centers for Disease Control and Prevention.
doi:http://dx.doi.org/10.15620/cdc.23447

[4]Nakatsuma A, Kaneda M, Kodama H, et al. Detection of HIV-1 p24 at Attomole Level by Ultrasensitive ELISA with Thio-NAD Cycling. PLoS One. 2015 Jun 22;10(6):e0131319. doi:10.1371/journal.pone.01 31319.

[5]TRUSTline HIV-1 Ab/Ag 4th Gen Rapid Test;PI- AR0018C Rev. C/01.09.2022; Athanese-DX., Chennai, India