HIV-1 p24 Sample Kit (Catalog #9282)
Suitable for developing a highly sensitive and specific HIV-1 p24 immunoassay

Meridian Life Science, Inc. offers a convenient sampling kit that contains the company’s best HIV-1 p24 reagents. These high-performing antigens and antibodies are used in the development of highly sensitive and specific HIV-1 p24 sandwich immunoassays.

Additional HIV antigens to HIV-1 gp41, HIV-1 gp160, HIV-1 gp120 and HIV-2 p36 are also available to complement the development of commercial HIV diagnostic assays. Please visit www.meridianlifescience.com to view the full range of HIV products and learn more about Meridian’s 30+ years of experience in manufacturing antigens and antibodies designed for commercial diagnostic assay applications.

INTRODUCTION

In 1985, the FDA approved the first HIV test (an enzyme-linked immunosorbent assay (ELISA) test kit) to screen for antibodies to HIV from serum. It was based on the detection and quantification of antibodies against specific HIV proteins and glycoproteins (e.g. p24, gp41, gp120) and was coined the “HIV Antibody Test”. This test is still the most common screening method for HIV and it is available in a variety of testing formats such as Western Blot or rapid assays, and newer 4th generation versions that incorporate the simultaneous detection of antibody and p24 antigen.

Several host and viral markers that occur during HIV infection are commonly used to identify infection and appear fairly consistently between different individuals at various times after infection. HIV-1 p24 antigen is a 24 kilodalton protein, which is a major structural component of the HIV-1 virus and is detectable earlier than HIV antibody during acute infection. The p24 antigen occurs early in infection due to the initial burst of virus replication and it is associated with high levels of viremia during which the individual is highly infectious. When detected, p24 antigen is highly specific for infection and can be of value in: (1) detecting early HIV infection, (2) screening blood, (3) diagnosing infection in the newborn, and (4) monitoring antiviral therapy. The introduction of the HIV p24 antigen test has enabled new-generation HIV assays to detect infection 2 weeks earlier (3-4 weeks after infection) compared to the early-generation HIV antibody tests (6-12 weeks after infection).

KIT COMPONENTS

ANTIBODIES: MAb to HIV-1 p24
1 vial each containing 0.25mg
Monoclonal antibody raised against the HIV-1 virus. Produced in ascites and purified by Protein A chromatography. >90% pure.
Cat# C65653M Concentration: 4-5 mg/mL (OD280nm, E0.1% = 1.3)
Cat# C01655M Concentration: 5.64 mg/mL (OD280nm, E0.1% = 1.4)
Cat# C01657M Concentration: 5.56 mg/mL (OD280nm, E0.1% = 1.4)
Cat# C65690M Concentration: 3.79 mg/mL (OD280nm, E0.1% = 1.3)

ANTIGEN: Recombinant HIV-1 p24
1 vial containing 0.25mg
Cat# VTI340 Concentration: 2 mg/mL (BCA)

ANTIBODY PAIRING COMBINATIONS

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METHODS

Below is a suggested sandwich ELISA protocol. Working dilutions must be determined by the user; however, suggested starting ranges are 1:20-1:200 for ELISA. Conjugation of the detection antibody must be performed in advance – in the protocol below a 1mg/mL stock of biotinylated detection antibody was used.

ELISA Procedure:

Dilution Buffer/Blocking Buffer: 1% BSA in PBST
Wash Buffer: PBST (PBS + 0.05% Tween-20)

1. 100 µL of 1 µg/mL capture antibody solution in carbonate buffer was applied to each well of a 96 well plate.
   The plate was incubated overnight at 2-8°C.
2. The plate was washed 3 times with 300 µL PBST/well, blocked for 1 hour with 300µL blocking buffer/well, and washed 3 times with 300 µL PBST/well.
3. 100 µL of 500 ng/mL antigen was titered 2.5-fold for a total of 11 data points. The plate was incubated 1 hr.
4. The plate was washed 3 times with 300µL PBST/well.
5. 100 µL of 0.1 µg/mL biotinylated detection antibody solution was applied to the plate. The plate was incubated 1 hr.
6. The plate was washed 3 times with 300µL PBST/well.
7. Thermo Fisher Streptavidin-poly HRP was diluted 1:10,000 in PBST and 100 µL of the diluted solution was added to each well.
   The plate was incubated for 1 hr.
8. The plate was washed 3 times with 300 µL PBST/well
9. 100 µL of TMB substrate (SurModics Cat#: TMBW-1000-01) was added to the plate and incubated for 10 minutes.
10. 100 µL of 0.16 M Sulfuric Acid solution was added to each well to stop the HRP-TMB reaction.
11. The plate was read at 450 nm using a 96 well plate reader.

SHIPPING & STORAGE

This product should be shipped on gel packs and stored at -20°C until ready for use. Avoid repeated freeze-thawing by storing multiple aliquots at -20°C.

References:
UCSF HIV InSite Knowledge Base Chapter (2014). Centers for Disease Control and Prevention (2014). Advantages and disadvantages of different types of FDA-approved HIV immunoassays used for screening by generation and platform