# Human Immunodeficiency Virus 1+2 (HIV) Antibody (HIV(1+2)-Ab) ELISA Kit by Abbexa

Devi Oktafiani[1]

[1]Universitas Airlangga

Sep 23, 2019

1 Works for me

dx.doi.org/10.17504/protocols.io.7gyhjxw

Devi Oktafiani

ABSTRACT

The human immunodeficiency virus (HIV) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS). AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype. In most cases, HIV is a sexually transmitted infection and occurs by contact with or transfer of blood, pre-ejaculate, semen, and vaginal fluids. Non-sexual transmission can occur from an infected mother to her infant through breast milk. An HIV-positive mother can transmit HIV to her baby both during pregnancy and childbirth due to exposure to her blood or vaginal fluid. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

EXTERNAL LINK

https://www.abbexa.com/human-immunodeficiency-virus-12-hiv-antibody-elisa-kit

manual_abx364861.pdf

1 Sample - Whole blood from plain tube (red cap) collection 3 mL - Centrifuge at approximately 1000 × g for 15 min.

2 Wash buffer Dilute the concentrated Wash buffer 20-fold (1/20) with distilled water (i.e. add 25 ml of concentrated wash buffer into 475 ml of distilled water). B. Assay Procedure Equilibrate the kit components and samples to room temperature for at least 30 minutes prior to use.

3 Determine the number of wells to be used. Any strips that are not being used should be kept dry and stored at 4°C.

4 Set up three Negative Control wells with 50 µl of Negative Control per well. Set up two Positive Control wells, one with 50 µL of HIV-1 Positive Control and the other with 50 µL of HIV-2 Positive Control. Set up one blank well with no solution. Add 50 µL of sample to each sample well. Add the solution at the bottom without touching the sides of the well. Shake the plate gently to mix the contents.

5 Seal the plate with a cover and incubate at 37°C for 60 min.

6 Remove the cover and discard the plate contents by tapping the plate on absorbent filter papers or other absorbent material.

7 Wash the plate 5 times with wash buffer. Do not let the wells completely dry at any time Manual Washing: Discard the solution without touching the side walls and wash the plate five times. Fill each well completely with Wash buffer and incubate on an ELISA shaker for 2 min. Invert the plate each time and decant the contents; tap it 4-5 times on absorbent paper towel to completely remove the liquid.

8 Add 50 µl of HRP-conjugate working solution into each well (except the blank wells). Add the solution at the bottom of each well without touching the side wall.

9 Seal the plate with a cover and incubate at 37°C for 30 min.

10 Wash the plate 5 times with wash buffer. Do not let the wells completely dry at any time.

11 Add 50 µl of TMB substrate A and 50 µl of TMB substrate B into each well. Cover the plate and incubate at 37°C in dark conditions for 15-20 minutes (incubation time is for reference only, do not exceed 30 minutes). When an apparent gradient appears in the positive control wells the reaction can be terminated.

12 Add 50 µl of Stop solution into each well (including the blank well). There should be a color change to yellow. Gently tap the plate to ensure thorough mixing

13 Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the absorbance at 450 nm immediately. C. Analysis

14 Calculations: Mean absobance of the positive control should be ≥ 0.80. Mean absobance of the negative control should be ≤ 0.08. CUT OFF value (Negative control < 0.05) = 0.05 + 0.10 = 0.15 CUT OFF value (Negative control ≥ 0.05) = Negative control + 0.10

15 Interpretation of results: If the postive control value is ≥ 0.80, and negative control value is ≤ 0.08, the test is valid, otherwise, the test is invalid. Samples: If O.D. of samples < CUT OFF, the test samples are considered negative. If O.D. of samples ≥ CUT OFF, the test samples are considered positive.