



Aug 17, 2020

Indirect Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HIV Antibodies in Human Serum

Norma McFarlane-Anderson¹¹University of the West Indies, Mona**1** Works for me dx.doi.org/10.17504/protocols.io.bjtkknkw

University of the West Indies

angel.vaillant@sta.uwi.eduAngel Justiz-Vaillant
University of the West Indies St. Augustine

ABSTRACT

This protocol was already used successfully to detect anti-HIV antibody in the serum of women with cervical dysplasia or cervical cancer in Jamaica, West Indies [1].

Reference

1. Justiz Vaillant A, Bazuaye PE, McFarlane-Anderson N, Smikle MF et al. Seroprevalence of Anti-HIV Antibodies in Women with Abnormal Pap Smears in Jamaica. British Journal of Medicine & Medical Research 2013, 3(4): 2197-2202.

DOI

dx.doi.org/10.17504/protocols.io.bjtkknkw

PROTOCOL CITATION

Norma McFarlane-Anderson 2020. Indirect Enzyme Linked Immunosorbent Assay (ELISA) for Detection of Anti-HIV Antibodies in Human Serum. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bjtkknkw>

LICENSE

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Aug 16, 2020

LAST MODIFIED

Aug 17, 2020

PROTOCOL INTEGER ID

40524

DISCLAIMER:

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

- 1 The 96 well polystyrene microplate (U-shaped bottom; Sigma-Aldrich) is coated with 50 ng of a mixture of synthetic peptides (including the fragment 579-601 of the HIV gp41 and fragments 254-274, 308-331 and 421-438 of the HIV gp120) for 4 h at 37°C.
- 2 The microplate is blocked with 3% non-fat milk in PBS, 25 µl/well, 1h at room temperature (RT).
- 3 The microplate is washed 4X with PBS-Tween-20.
- 4 Duplicates of 25 µl of 1:16 diluted human sera are added.
- 5 After incubation for 90 min at RT the microplate is washed 4X with PBS-Tween 20.
- 6 Then, 25 µl of a chimeric commercially-prepared recombinant protein LA-HRP conjugate (Sigma-Aldrich) diluted 1:5000 is added.
- 7 After incubation for 90 min at RT and rewashing steps 25 µl TMB is added to each well for 15 min in the dark.
- 8 The reaction is stopped with 3M H₂SO₄.
- 9 The microplate is read in a microplate reader at 450 nm.
- 10 In the ELISA is included a pooled human sera with high titre of anti-HIV antibodies as positive control, a pooled sera from healthy individuals as negative control and 0.9% normal saline solution was used as the blank.
- 11 The cut-off point is calculated as mean optical density (XOD) of negative control plus two standard deviation (SD).