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# Inhibition immunoassay of the HIVgp120\_anti-HIVgp120 (Ab-1) reaction by anti-anti-idiotypic-HIVgp120 antibody (Ab-3).

Angel A Justiz-Vaillant<sup>1</sup><sup>1</sup>University of the West Indies St. Augustine

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Works for me

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University of the West Indies

[angel.vaillant@sta.uwi.edu](mailto:angel.vaillant@sta.uwi.edu)

Angel Justiz-Vaillant

University of the West Indies St. Augustine

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MATERIALS

NAME	CATALOG #	VENDOR
Carbonate Coating Buffer	421701	BioLegend
Anti-Chicken IgY, HRP Conjugate, 300ul	G1351	Promega
PBS Blocking buffer with non fat milk	SB0625.SIZE.1pk	Bio Basic Inc.
96-Well Microtiter <sup>®</sup> ; Microplates, Polystyrene, 400 $\mu$ m; L, Nonsterile, U-bottom	2205	Thermo Fisher
TMB Substrate Set	421101	BioLegend
Fragment 254-274 of Gp120 of HIV (peptide)		

- 1 Ninety six well polystyrene microplate (U-shaped bottom, Sigma-Aldrich Co, St Louis Missouri) is coated with 50  $\mu$ l/well of 1 ng/ $\mu$ l solution of gp-120 peptide, fragment 254-274 (Sigma) in carbonate-bicarbonate buffer pH 9.6 (Sigma) for 4 hours at 37°C.
- 2 The microplate is then washed 4 times with PBS Tween-20.

- 3 The microplate is blocked with 3% non-fat milk in PBS, 25 µl/well, 1h, RT.
- 4 Triplicates of serial doubling dilutions of cat serum diluted in PBS-3% non-fat milk, pH 7.4 are added to the microplate which is then incubated for 90 min at 37°C.
- 5 The microplate is washed 4 times with 150 µl/well of PBS-Tween 20, and 25 µl of a water soluble fraction (WSF) containing anti-HIV-gp120 antibodies (titer =1:1024) is added to each well.
- 6 After adding WSF and it follows an incubation period (90 min, 37°C).
- 7 The microplate is washed 4 times and 25 µl of a 1:30,000 dilution of rabbit anti-chicken IgY-HRP (Sigma-Aldrich Co) is added to each well.
- 8 The microplate is then incubated at 37°C for 1 h.
- 9 The microplate is washed with PBS-Tween 20, 4 times.
- 10 Add 25 µl of TMB to the each well of the microplate and incubate the plate in the dark for 15 min.
- 11 The reaction was stopped with 3M H<sub>2</sub>SO<sub>4</sub>.
- 12 The microplate is read at 450 nm in a microplate reader.