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ELISA for Detection of HTLV-I and HTLV-II Positive Sera

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Reagents

HTLV-I/II Peptides	HTLV-I Peptides Env-1, Env-5, Gag-1a (Catalog# 1459, 1461, 1463); HTLV-II Peptides Env-20, Env-2 (Catalog# 1462, 1460).
Carbonate Buffer	Sigma, Catalog# C3041, dissolve in distilled water.
PBS-T	PBS with 0.05% Tween-20
Blocking Solution	PBS-T with 3% BSA
Antibody Diluent	PBS-T with 1% BSA
Primary Antibody	HTLV-I/II test sera, prepared at 1:20 in antibody diluent.
Secondary Antibody	Goat anti-human IgG(Fc) alkaline phosphatase conjugate (Sigma, Catalog# A9544), prepared at 1:10,000 in antibody diluent.
PNPP Solution	p-Nitrophenyl phosphate (Sigma, Catalog# N2640). Dissolve 1 tablet per 5 ml in sodium carbonate buffer immediately prior to use.
Sodium Carbonate Buffer	1.69 g $NaHCO_3$, 3.51 g Na_2CO_3, 0.41 g $MgCl_2(6H_2O)$; add distilled water to bring volume to 2.0 L, adjust pH to 8.6.

Procedure

1. Coat polyvinyl plate (Immulon-II) with HTLV-I or HTLV-II peptides at 10 µg/ml, 50 µl per well, in carbonate buffer. Incubate plate overnight at 4°C.
2. Wash plates six times in PBS-T.
3. Add 150 µl of blocking solution to each well. Incubate plate for 2 hours at room temperature.
4. Wash plates six times in PBS-T.
5. Add 50 µl of diluted serum to each well. Incubate plate overnight at 4°C.
6. Wash plate six times in PBS-T.
7. Add 50 µl of diluted secondary antibody to each well. Incubate plate for 2 hours at room temperature.
8. Wash plate six times with PBS-T.
9. Add 50 µl PNPP to each well and incubate at room temperature.

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10. Read the plate at 405 nm as the yellow color approaches an OD of 1.0 in the positive control and test samples. Seropositivity in the test samples is defined as +2 standard deviations over the normal control mean.