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DATA SHEET

Reagent:	Anti-HIV-1 Polyclonal (IgA from long-term survivor)
Catalog Number:	12046
Lot Number:	120169
Provided:	400 µL purified IgA at 100 µg/mL, in 10mM sodium phosphate pH 7.4, 137 mM NaCl, 2.7 mM KCl.
Host or Host Site:	IgA from human infected with subtype B HIV-1 for 22 years with minimal anti-retroviral therapy.
Titer:	Total protein is 100µg/mL using bicinchoninic acid protein assay. IgA neutralizes subtype B HIV strain 92BR021 and subtype C HIV strain 97ZA009 (primary isolate) with an IC50 value respectively of 0.7 and 1.0 µg/mL using pooled human peripheral blood mononuclear cells (PBMC).
Description:	The polyclonal IgA is from a subject infected with HIV-1 for 22 years. Antibodies directed against multiple epitopes are present. The antibody species responsible for the observed cross-subtype neutralizing activity are directed to the gp120 421-433 epitope. Purity is >90%, determined from silver stained denaturing gel electrophoresis.
Special Characteristics:	This antibody preparation does not neutralize pseudovirion infection of the TZM-bl cell line. It neutralizes infection of the PHA-activated human PBMCs by genetically diverse primary strains [belonging to subtypes A, B, C, D and AE].[1] Neutralization assays are conducted as in ref 2. Briefly, human PBMCs are pooled from 4 non-HIV-infected donors and activated with 5 µg/mL phytohemagglutinin for 3 days. Virus stock is diluted in "RPMI medium" (RPMI 1640,antibiotics, 5% IL-2, 20% FBS). A primary isolate (for example, CCR5-dependent subtype C 97ZA009, 100 $TCID_{50}$ per well) is incubated for 1 hour with antibody diluted 1:2 in RMPI medium, and diluted further with 1:1 RPMI:PBS mixture. The mixture is then added into the wells containing 250,000 PBMCs in RPMI medium, and incubated for 3 days. Cells are washed and intracellular p24 is measured by ELISA on day 4. Inter-assay variability of neutralization was determined in 9 repeat assays using the subtype C virus ZA009. Neutralization was detectable in 9 out of 9 assays. The IC50 values varied from 0.07-3.4 µg/mL. Endotoxin concentration in stock IgA solution is 0.43 EU/mL. The endotoxin concentration will be 0.0043 EU/mL at final concentration in the neutralization assay equivalent to the IC50 (1µg/mL). Binds electrophilic 416-433 peptide detectably at 100 µg/mL.

Cross-reactivity has not been studied.

Recommended Storage: Keep at 4°C for short term storage and -80°C for long term storage. Avoid freeze-thaw cycles as reagent degradation may result.

Contributor: Drs. Sudhir Paul and Stephanie Planque.

References:

1. Planque S, Salas M, Mitsuda Y, Sienczyk M, Escobar MA, Mooney JP, Morris MK, Nishiyama Y, Ghosh D, Kumar A, Gao F, Hanson CV, Paul S. Neutralization of genetically diverse HIV-1 strains by IgA antibodies to the gp120-CD4-binding site from long-term survivors of HIV infection. *AIDS*. 2010 Mar 27;**24**(6):875-84. Abstract

2. Paul S, Karle S, Planque S, Taguchi H, Salas M, Nishiyama Y, Handy B, Hunter R, Edmundson A, Hanson C. Naturally occurring proteolytic antibodies: selective immunoglobulin M-catalyzed hydrolysis of HIV gp120. *J Biol Chem*. 2004 Sep 17;**279**(38):39611-9. Abstract

NOTE: Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH: Anti-HIV-1 Polyclonal (IgA from long term survivor) from Drs. Sudhir Paul and Stephanie Planque." Also include the references cited above in any publications.

Limited to 1 aliquot per lab.

Reagent must not be used or incorporated for commercial purposes.

Patent Pending.