Technical Data Sheet

WR304 ANTIBODY FOR HIV AND LIPID RESEARCH

Avanti No.	Description	No. of Assays
330021S	WR304 monoclonal	100
330022S	WR304 monoclonal biotinylated	100

Background:

The WR304 antibody was produced by the Walter Reed Army Institute of Research (WRAIR) using an advanced, targeted Lipid-A adjuvant protocol[i]. The antigen was a lipid, brain PI(4)P[i].WR304 is a polyvalent antibody that binds specifically to PIP, PIP2, sulfatide, DMPA, cholesterol, squalene and Lipid A[ii]. It neutralizes infectious HIV-1 virus[iii]. WR304 is the first of a series of targeted antibodies that Avanti® Lipodomics will produce under license from WRAIR.

Antibody Information:

Antigens: Brain PI(4)P.
Ig Class: Mouse IgM (kappa).
Specificity: WR304 recognizes PIP, PIP2, sulfatide, DMPA, cholesterol, squalene and Lipid A.
Antibody Source: Monoclonal antibody from BALB/c-derived hybridoma WR304.
Production: In vitro cell culture.
Purification: Ultra filtration through 100 KDa cut-off filters.
Purity: ≥ 95%.
Formulation: WR304 is provided as a sterile-filtered solution in Tris buffered saline (TBS). WR304 binding to porcine brain PI(4)P is inhibited by high concentration of Ca^{2+} (10 mM)[iv] and addition of 1 mM EDTA stimulates this binding activity. PBS and other phosphate-containing buffers should not be used as they inhibit binding to the phospholipid head group[iii].
Mass and Concentration: Refer to Product Label.
Recommended Applications: ELISA, IHC, Dot Blot, Flow cytometry and HIV-1 infectivity.
Storage conditions: Store undiluted at either -20°C or -80°C.
Hazardous/Non-hazardous Components: This product contains no substances that, at their given concentration, are known to be hazardous to health. Therefore, there is no MSDS for this product.

Product use:

The WR304 antibody has been used for the quantitation of PIP in direct ELISA and Dot Blot analysis. The WR304 antibody specifically binds to PIP[i] and inhibits the infectivity of HIV-1 to peripheral blood mononuclear cells (PBMC)[iii]. A biotinylated form of WR304 is used for enzyme-linked immunosorbent assay (ELISA) determination of PIP in serum or plasma samples. A TopFluor™-conjugated WR304 antibody will be available for immunohistochemistry (IHC) and flow cytometry.

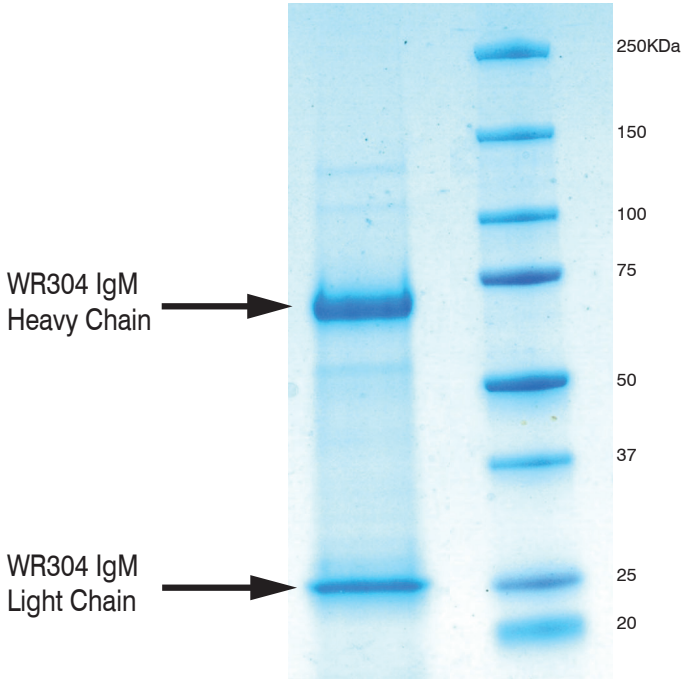

Fig. 1 SDS-PAGE gel of purified WR304 antibody. The antibody was reduced with β-mercaptoethanol.

Note:

After thawing, centrifuge this product at > 1,000 g for 5 minutes to collect any antibody solution that may be retained in the cap.
The recommended long-term storage for WR304 is at -80°C. After initial thawing remaining product should re-frozen at -80°C.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

References:

[i]Wassef NM, Roerdink F, Swartz GM Jr, Lyon JA, Berson BJ,Alving CR. Phosphate-binding specificities of monoclonal antibodies against phosphoinositides in liposomes. *Mol. Immunol.*(1984) 21: 863-868.
[ii]Matyas GR, Beck Z, Karasavvas N, Alving CR. Lipid binding properties of 4E10, 2F5, and WR304 monoclonal antibodies that neutralize HIV-1. *Biochim. Biophys. Acta* (2009) 1788: 660-665.
[iii]Brown BK, Karasavvas N, Beck Z, Matyas GR, Birx DL, Polonis VR, Alving CR. Monoclonal antibodies to phosphatidylinositol phosphate neutralize human immunodeficiency virus type 1: role of phosphate-binding subsites. *J Virol.* (2007) 81:2087-2091
[iv]Beck Z, Karasavvas N, Tong J, Matyas GR, Rao M, Alving CR. Calcium modulation of monoclonal antibody binding to phosphatidylinositol phosphate. *Biochem. Biophys. Res. Commun.* (2007) 354: 747-751.

APPLICATIONS:

1. Direct ELISA Protocol

96 well "U" bottom Immulon 2HB plates (Thermo 3655were used for lipid ELISA. Stock solutions of brain PI(4)P in $CHCl_3$:MeOH:H_2O (20:9:1) were diluted in methanol to 10 μM. 1 nmol in 100 μL was added to each well and the solvent was allowed to evaporate O/N[ii]. The plates were blocked with 0.3% Gelatin in TBS (0.8% NaCl, 20 mM Tris-HCl pH 7.4, Mediatech) containing 1 mM EDTA for 1h at room temperature[iv]. The wells were washed four times with TBS containing 1 mM EDTA. 10μL aliquots of a serial dilution the WR304 antibody (400-1.6 ng/100 μL) in TBS containing 0.3% Gelatin, 1 mM EDTA were added to each well and incubated for 1h at RT. The amount of IgM bound to each well was quantified with goat anti-mouse IgM-HRP. After 1h incubation at room temperature, unbound secondary was washed off with TBS. ABTS (KPL, Inc.) was added as substrate and absorbance was read at 405 nm with a correction for light scattering at 650 nm.
Alternatively, using 96 well "U" bottom Microfluor 1 white plates (Thermo 6905) with the above protocol, the amount of IgM bound to each well was quantified with goat anti-mouse IgM-AP (Sigma) at a 1:30,000 dilution and a LumiPhos 530 substrate after 1hr incubation at room temperature (Fig 2).

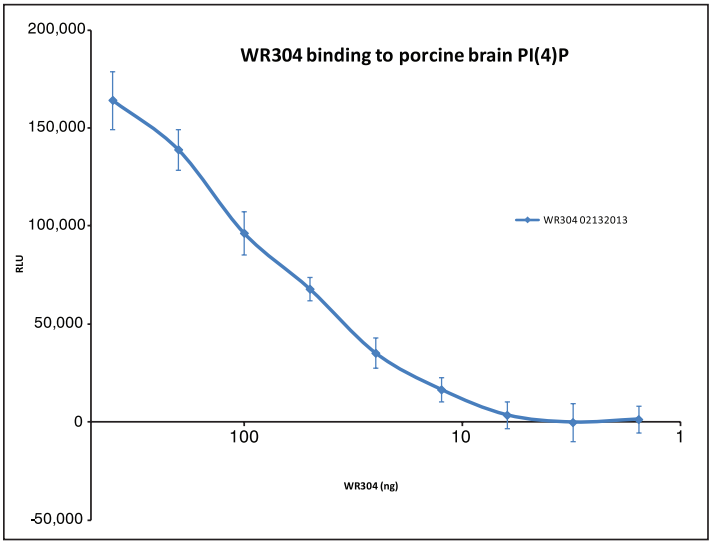

Fig. 2. Sample Direct ELISA
Immobilized antigen, porcine brain PI(4)P probed with WR304 antibody followed by a GAM IgM-AP. Bound WR304 was measured as AP-dependent luminescence using LumiPhos 530 as substrate.

2. Dot Blot Protocol

The WR304 antibody was used to probe an Avanti Inositol Snooper™, with a range of phosphatidyl inositol lipids on nitrocellulose membranes (Fig. 3). 1 μg (~1 nmol) of each lipid was sprayed on nitrocellulose (GE Hybond-C Extra). The membranes were then blocked with 3% BSA (fatty acid free) in TBS (0.8% NaCl, 20 mM Tris-HCL pH 7.4) containing 1 mM EDTA. The blocked membranes were then probed with WR304 (1.0 μg/mL) in TBS containing 1% BSA and 1 mM EDTA for 1 h at RT. After washing the membranes 3 times with TBS to remove unbound WR304 antibody, the bound antibody was detected with goat anti-mouse IgM conjugated to horseradish peroxidase (HRP) (Southern Biotech) at a 1:5,000 dilution in TBS containing 1% BSA, 1 mM EDTA for 1 h at RT. After washing the membranes 3 times to remove unbound secondary antibody, bound HRP was visualized on an X-ray film with an enhanced chemiluminescence substrate (Pierce).

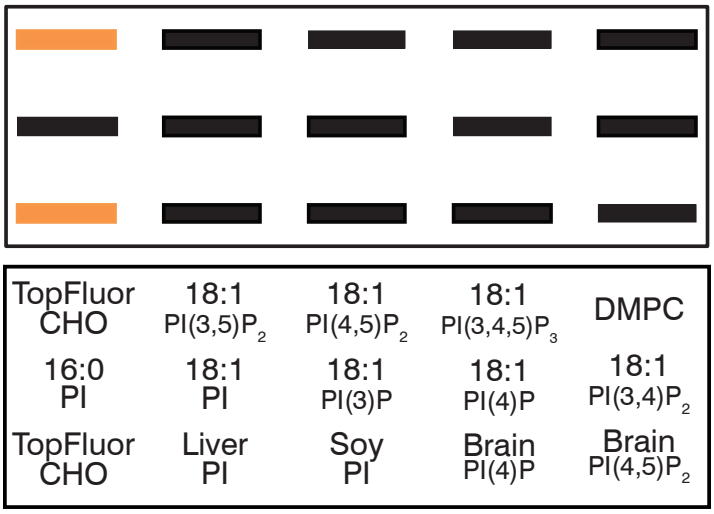

Fig. 3. Avanti Inositol Snooper™
Grid showing the distribution of 1 μg samples of DMPC, 18:1 PI, 18:1 PI(3)P, 18:1 PI(5)P, 18:1 PI(3,4)P2, 18:1 PI(3,5)P2, 18:1 PI(4,5)P2, 18:1 PI(3,4,5)P3, bovine liver PI, soy PI, porcine brain PI(4)P and porcine brain PI(4,5)P sprayed on nitrocellulose. Top-Fluor labeled cholesterol (TopFluor-CHO) was included as a fluorescent lipid marker. These immobilized lipids were probed with a WR304 (1 μg/mL) followed by a goat anti-mouse IgM-HRP secondary antibody.

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