# Monoclonal Antibody Directed against Human Type V Phospholipase A2 (hVPLA2)

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## Technology description

Hybridoma F66-3G1.3 (Human group V phospholipase A2) UCHI 0822
Antigen:Human group V phospholipase A2
Antigen Background:Phospholipases a2 (PLA2) are a superfamily of heterogeneous enzymes that hydrolyze the fatty acid esterified at the sn-2 position of glycerophospholipids. Two major classes of mammalian $Ca^{2+}$ dependent PLA2s, 85 kDa cytosolic PLA2 (cPLA2), and 14 kDa secretory PLA2 (sPLA2), have been well characterized and cloned. At least six isoforms of sPLA2s have been identified in mammals; they include group Ib (pancreatic), IIa, IIc, IId, V, and X. Among these sPLA2s, group IIa PLA2 has been implicated in receptor coupled arachidonate (AA) release in many inflammatory cells because of its increased concentration in the serum and exudates in certain inflammatory diseases. Group V PLA2 was detected in mouse macrophage-like P388D1 cells and murine mast cells, in which it acts as the primary effector enzyme in the formation of AA metabolites.
Antibody Class:Mouse IgG1
Phospholipase Species Recognized:Human and mouse.
Hybridoma Production:Three Balb/c mice were each immunized intraperitoneally with 70 m g of pure W79A hVPLA2 in phosphate-buffere d saline (PBS) with 1:1 complete Freund's adjuvant (Sigma). At Day 14, all mice were injected intraperitoneally with additional 70 mg of W79A hVPLA2 emulsified with incomplete Freund's adjuvant. The mice were bled at Day 32, and the serum was screened for the presence of hVPLA2 antibody by solid phase enzyme-linked immunoadsorbent assay (ELISA). Seven days later (Day 39), the animals were given an additional boost of 70 m g W79A hVPLA2, and at Day 52, the serum was again tested for its specificity to hVPLA2 by ELISA. For animals that presented strong immunoreactivity to hVPLA2, a final boost of 25 m g W79A hVPLA2 was administered intravenously, and these animals were sacrificed 3 days later. Immediately, the spleen was harvested and cultured as previously described. (Yokoyama et al., 1991) The splenocytes were fused with mouse myeloma cell line s/p20-Ag14 according to the standard method. Hybridomas secreting MAb were screened by ELISA on immobilized hVPLA2 and hIIaPLA2. Normal mouse serum, and fusion mate, s/p20-Ag14 were used as negative controls; hVPLA2-injected mouse serum was used as a positive control. Specific hybridomas designated as MCL-3G1, MCL-2A5, and MCL-1B7 were grown in growth media containing 15% fetal calf serum (FCS) by the limiting dilution method, and specificity for hVPLA2 was again tested as above.

Isotyping of hybridoma supernatant was performed according to the manufacturer's instruction with the Isostrip Mouse Monoclonal Antibody Isotyping Kit (Boehringer Mannheim, Lewes )

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