OriGene Technologies Inc.

9620 Medical Center Drive, Ste 200
Rockville, MD 20850
UNITED STATES
Phone: +1-858-888-7900
Fax: +1-858-888-7904
US-info@acris-antibodies.com

OriGene EU

Acris Antibodies GmbH
Schillerstr. 5
32052 Herford
GERMANY
Phone: +49-5221-34606-0
Fax: +49-5221-34606-11
info@acris-antibodies.com

Monoclonal Antibody to Sulfotyrosine - Aff - Purified

Catalog No.: AM32522SU-N

Quantity: 30 µl

Background: Sulfotyrosine is a common post-translational modification of secretory, plasma membrane, and lysosomal proteins. The sulfation reaction is catalyzed by specific sulfotransferase enzymes in the trans-Golgi-network. Tyrosine sulfation occurs in all multicellular organisms and up to 1% of the tyrosines in the total protein content of a cell can be sulfated. Sulfotyrosines mediates many receptor-ligand binding interactions, including the interaction of P-selectin glycoprotein ligand-1 with P-selectin, factor VIII binding to von Willebrand factor, and interactions between thrombin and hirudin. Sulfotyrosine is also involved in the entry of several parasites and viruses; for example, tyrosine sulfation of the HIV-1 co-receptor CCR5 is required for viral entry into host cells, and tyrosine sulfation of the Duffy antigen/receptor for chemokines is crucial for erythrocyte invasion by the malaria parasite Plasmodium vivax.
This clone was derived from the antibody described in Kehoe *et al.*, 2006.

Host / Isotype: Mouse / IgG2a

Clone: Sulfo-1C-A2

Immunogen: This antibody was developed using a phage display library as described in Kehoe et al., 2006. The selection antigens were KAKISDP-DY(SO3)MTGYMDAC and KDKKYATEY(SO3)-EYLDYDFC. Epitope: Sulfated Tyrosine.

Format:
State: Liquid purified Culture Supernatant
Purification: Protein G Affinity Chromatography
Buffer System: 100mM Tris-glycine, 150mM NaCl, pH 7.4
Preservatives: 0.05% Sodium Azide

Applications:
Western Blot: Bovine Fbrinogen was treated with 1.5 mg/ml abalone sulfatase at 37°C overnight. The protein was resolved by electrophoresis, transferred to PVDF and probed with anti-Sulfotyrosine Antibody (Cat.-No AM32522SU-N, Lot#DAM1483552, 1/1000 dilution). Protein was visualized using a Goat anti-Mouse secondary antibody (1/2000) conjugated to HRP and chemilumnescent detection (See **Figure 1**).
Various cell lysates were resolved on a previous lot by electrophoresis, transferred to PVDF and probed with antisulfotyrosine, 1/1000 dilution, or a control IgG. Proteins were visualized using Goat anti-Mouse secondary antibody (1/2000) conjugated to HRP and chemilumnescent detection (See **Figure 2**).
Immunoprecipitation: The antibody was shown to precipitate sulfotyrosine-containing proteins in NIH 3T3 cell lysates by immunoprecipitation. Lysates were precipitated using either anti-sulfotyrosine or an isotype control monoclonal, then detected by western blotting using the sulfotyrosine antibody (data not shown).
ELISA: Previous lots have been shown by competitive ELISA to recognize peptides containing tyrosines that were sulfated, but not unsulfated or phosphorylated (see Kehoe

et al., 2006) (data not shown).
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Recognizes sulfated Tyrosine residues on various proteins.
Does not recognize phosphotyrosine or unmodified Tyrosines.
The antibody is predicted to recognize any Sulfotyrosine, regardless of surrounding amino acid sequence.

Species Reactivity: **Tested:** Human, Mouse, Bovine and Porcine.

Storage: Store undiluted at 2-8°C.
Shelf life: one year from despatch.

General Readings:
1. Kehoe, J.W, et al., (2006). Mol. Cell. Proteomics. 5:2350-63.
2. Moore, K.L., et al., (2003). J. Biol. Chem. 278: 24243-46.
3. Seibert C, Sakmar TP. (2007). Biopolymers, in press.
4 Yu, Y., et al., (2007). Nature Methods. 4:583-88.

Protocols:

Western Blotting

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1 µg/mL each aprotinin, leupeptin, pepstatin; 1 mM Na3VO4; 1 mM NaF) and transfer the proteins to a PVDF membrane. Wash the membrance twice with water.
2. Block the blotted PVDF in Pierce super-block for 1 hour at room temperature with constant agitation.
3. Incubate the PVDF with 1/1000 dilution of anti-Sulfotyrosine Antibody Cat.-No AM32522SU-N, diluted in super-block for two hours at room temperature with agitation.
4. Wash the PVDF membrance three times with TBS-0.05% Tween 20.
5. Incubate the PVDF in the secondary reagent of choice (a goat anti-mouse HRP conjugated IgG was used) in TBS-5 %MLK for 1 hour at room temperature with agitation.
6. Wash PVDF with three times with TBS-0.05% Tween 20.
7. Use detection method of choice (VisualizerTM Spray and GlowTM)

Pictures:

Figure 2: Western blot with Various cell lysates:
Lanes 1,4: 3T3/NIH cell lysate.
Lanes 2,5: HEK 293 cell lysate.
Lanes 3,6: Bovine cardiac tissue lysate.

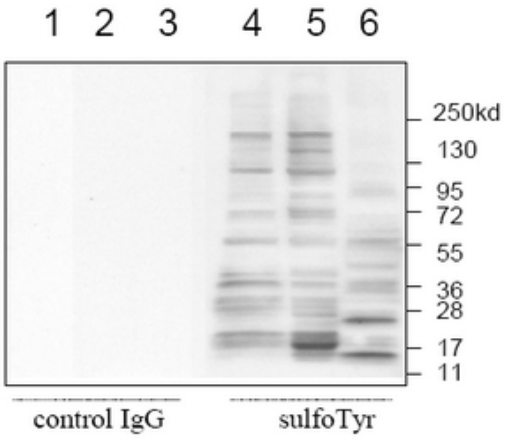

Figure 1. Western blot of purified bovine fibrinogen in the absence or presence of abalone sulfatase treatment, detected with anti-Sulfotyrosine Antibody, clone Sulfo-1CA2 Cat.-No AM32522SU-N.