custom antibodies & services

BIOTEM
885, rue Alphonse Gourju
38140 Apprieu
FRANCE

Tel: +33 (0)4 76 65 10 91
Fax: +33 (0)4 76 67 48 86

info@biotem.fr
www.biotem-antibody.com

TECHNICAL DATASHEET

S1P1 Monoclonal Antibody
Clone 2B9
Ref # BIO.020.2

Product description

Description	Mouse Anti-S1P1 Monoclonal Antibody
Host species	Mouse
Validated applications	WB, IP, IF, ELISA and FACS
Specificity	Monoclonal Antibody specific for Sphingosine 1-Phosphate Receptor 1
Species reactivity	Human, other species not tested

Target exploration

Sphingosine 1-Phosphate Receptor 1 (S1P1) is a multi-pass cell membrane protein that belongs to the G-protein coupled receptor superfamily (GPCR). S1P1 is a receptor for the lysosphingolipid sphingosine 1-phosphate (S1P). S1P is a bioactive lysophospholipid that elicits diverse physiological effect on most types of cells and tissues. This inducible epithelial cell G-protein-coupled receptor may be involved in the processes that regulate the differentiation of endothelial cells. Clinical significance of S1P1 encompasses various diseases including cancer and multiple sclerosis. S1P1 seems to be coupled to the G(i/o) subclass of heteromeric G proteins.

Other Names: S1P Receptor 1, S1PR1, Sphingosine 1-Phosphate Receptor 1, Endothelial differentiation G-Protein Coupled Receptor 1, EDG-1.

Properties

Form	Liquid or Lyophilized
Storage instructions	Store at +4ºC short term (1-2 weeks). Aliquot and store at -20ºC or -80ºC. Avoid repeated freeze / thaw cycles
Storage buffer	Phosphate Buffered Saline 10 mM – NaCl 0.15 M– pH 7.4.
Purity	Purified IgG fraction prepared by affinity chromatography on protein A
Isotype	IgG2a
Restrictions	For research use only

Applications

WB	Suggested dilution: 1/5 000
IP	Suggested dilution: 1/300
IF	Suggested dilution: 1/500
ELISA	Suggested dilution: 1/4 000 to 1/10 000
FACS	Suggested dilution: 1/100

Optimal dilutions/concentrations should be determined by the end user

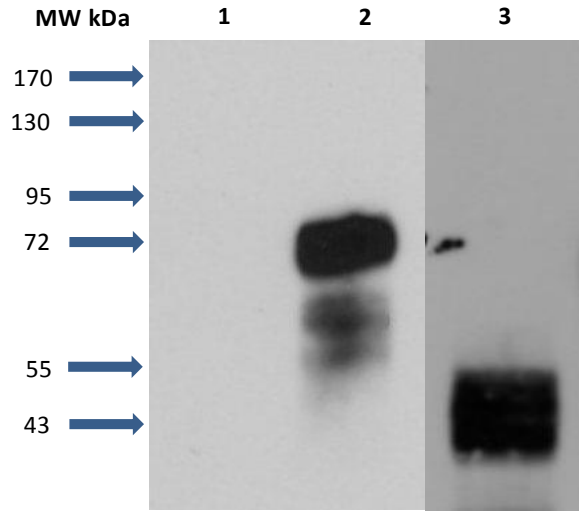

Fig1. Detection of S1P1 receptors in transfected cells and human umbilical vein endothelial cells (HUVEC)
(1) CHO-K1 cell membranes. (2) S1P1-GFP CHO cell membranes. (3) HUVEC membranes. CHO-K1 cells were stably transfected with S1P1-GFP construct. Samples were analysed by SDS-PAGE and immunoblotted with Mouse Anti-S1PR1 Monoclonal Antibody (clone 2B9) (1/5000 w/v dilution). S1P1 receptor was fused with the green fluorescent protein (GFP) at its C-terminus.
Molecular weights. GFP: 27 kDa, S1P1: 43 kDa, S1P1-GFP: 70 kDa.

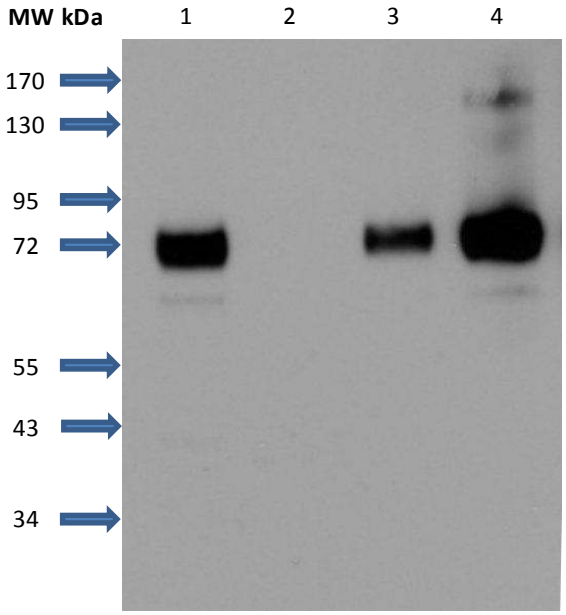

Fig.2 Immunoprecipitation of S1P1 receptors
S1P1-GFP receptors were immunoprecipitated with a mouse monoclonal antibody anti-S1P1 after solublization of membranes with NP40 detergent and interaction with sepharose-protein G phase. Detection of precipitated receptors was realized with a rabbit secondary anti GFP antibody coupled with HRP. (**1**) CHO-S1P1-GFP membranes. (**2**) Control without anti-S1P1 antibodies. (**3**) CHO-S1P1-GFP cell membranes solubilized with NP-40. (**4**) Immunoprecipitated sample. For control and Immunoprecipitated sample, sepharose-protein G was boiled in Laemmli buffer for 5 min at 100°C.

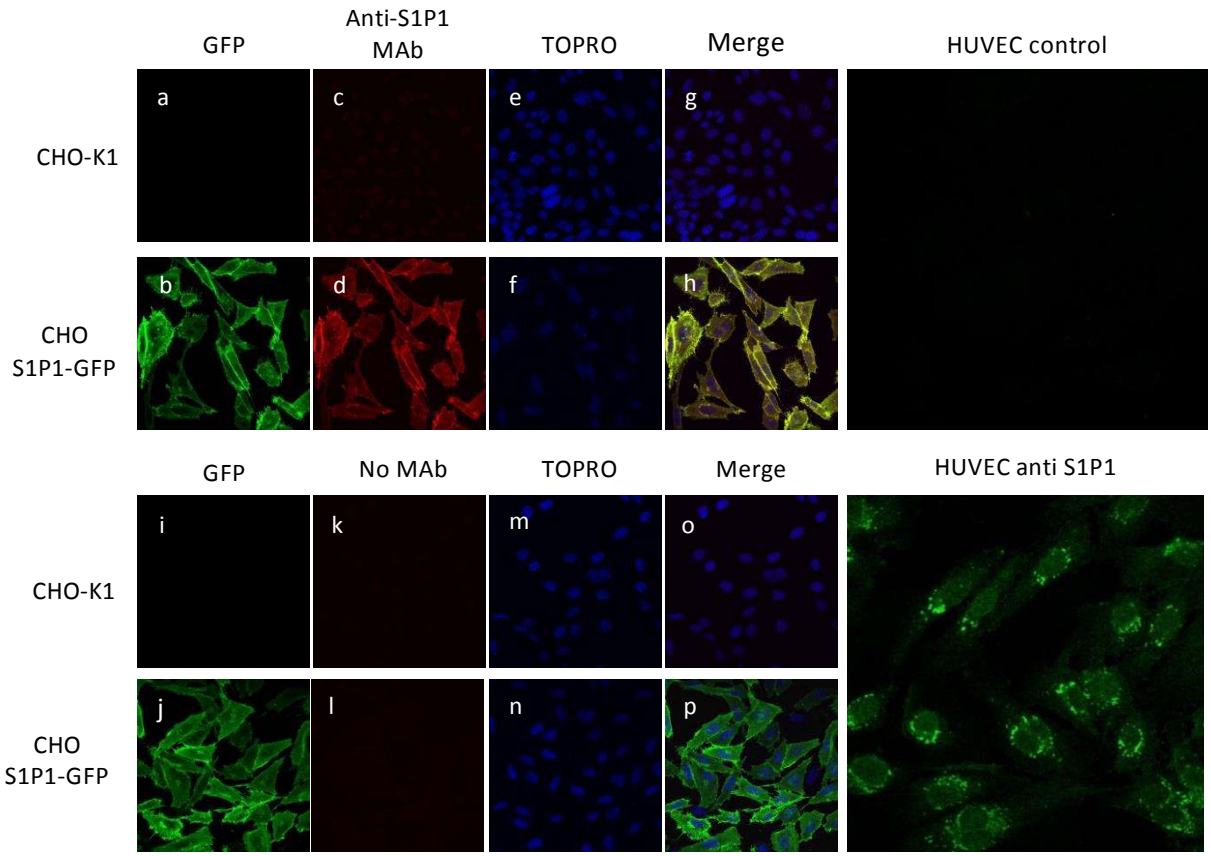

Fig3. Immunofluorescence detection of S1P1 receptors in transfected cells and HUVEC
Endogenous S1P1 receptors were detected by confocal microscopy in HUVEC and in transfected CHO cells stably expressing S1P1-GFP with an anti-S1P1 monoclonal antibody (1/100, w/v).
CHO-K1 cells were used as negative control. S1P1-GFP receptors were detected by fluorescence (excitation 488 nm, emission 500-550 nm) (a, b, i, j, green fluorescence) and immunofluorescence (Cy3-Ac II anti-mouse, excitation 559 nm, emission 570-625 nm) with an anti-S1P1 monoclonal antibody (c, d, k, l, red fluorescence). Nuclei were stained with TO-PRO-3 (excitation 635 nm, emission 655-755 nm, e, f, m, and n blue fluorescence). Merge images are presented (g, h, o, p).
HUVEC were stained with anti-S1P1 primary antibody and Alexa-488 secondary antibody (excitation 488 nm, emission 500-550 nm). HUVEC control was realized with omitting anti-S1P1 primary antibody.

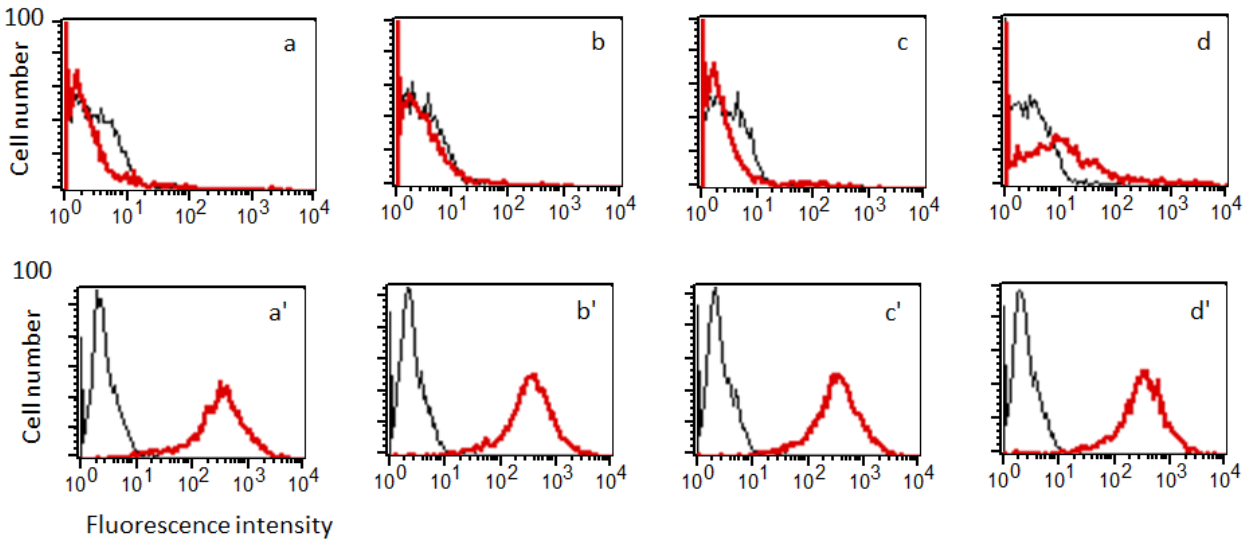

Fig4. Comparative evaluation of different Anti-S1P1 antibodies in flow cytometry
S1P1 receptors were detected with anti-S1P1 antibodies (1/100) by flow cytometry in recombinant CHO cells stably expressing S1P1-GFP (red line) and in CHO-K1 cells (black line) as negative controls.
S1P1-GFP receptors were detected by fluorescence (excitation 488 nm, emission 530/30 nm) (a', b', c', d') and immunofluorescence (APC, excitation 635 nm, emission 661/16 nm) with different anti-S1P1 antibodies (a-Mab1, b-Pab1, c-Pab2 and d-BIOTEM clone 2B9).