Rabbit (polyclonal) Anti-ETS1 [pSpS282/285] Phosphospecific Antibody, Unconjugated

PRODUCT ANALYSIS SHEET

Catalog Number: 44-1111G (10 mini-blot size)

Lot Number: See product label

Volume: 100 µL

Form of Antibody: Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without Mg^{2+} and Ca^{2+}), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier.

Preservative: 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.)

Purification: Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated ETS. The final product is generated by affinity chromatography using an ETS1-derived peptide that is phosphorylated at serines 282 and 285.

Immunogen: The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human ETS1 that contains both serine 282 and 285.

Target Summary: The proto-oncogene ETS1 (E-twenty-six-specific sequence) is the founding member of a family of transcription factors that share a highly conserved DNA-binding domain, called the ETS domain, which recognizes a nucleotide motif with a GGAA/T core sequence. The ETS genes are dispersed on two separate chromosomal loci called *ets-1,* which codes for a 54 kDa protein, and *ets-2,* which codes for a 56 kDa protein. They are responsible for the regulation of critical genes involved in cell proliferation, differentiation, lymphoid development, motility, invasion, angiogenesis, and apoptosis. ETS1 has been shown to be phosphorylated on serine 251, 257, 282, and 285 by calmodulin-dependent kinase II *in vitro* and following antigenic stimulation of T or B lymphocytes or treatment with calcium ionophores *in vivo*. Phosphorylation at these sites, which are adjacent to the DNA binding domain, has been shown to inhibit ETS1 binding to specific DNA sequences but does not affect ETS1 localization to the nucleus.

Reactivity: Human ETS1. Chicken, pufferfish (*Tetraodon fluviatilis*), frog (*Xenopus laevis*), rat (each 100% homologous) and mouse (91% homologous) have not been tested, but are expected to react.

Applications: The antibody has been used for Western blotting.

Suggested Working Dilutions: For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application.

Storage: Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient.

Expiration Date: Expires one year from date of receipt when stored as instructed.

Positive Controls Used: Jurkat cells treated with hydrogen peroxide, A23187, or anisomycin.

This product is for research use only. Not for use in diagnostic procedures.

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Related Products:

Antibodies:

ETS1 [pT^{38}] antibody, Cat. #44-1104G

ETS1 [pS^{251}] antibody, Cat. #44-1107G

ETS1 [pS^{282}] antibody, Cat. #44-1109G

ELK1 [pS^{383}] antibody, Cat. #44-238G

c-Fos [pT^{232}] antibody, Cat. #44-280G

References:

Liu, H., et al. (2004) AML1/Runx1 recruits calcineurin to regulate granulocyte macrophage colony-stimulating factor by Ets1 activation. J. Biol. Chem. 279:29398-29408.

Liu, H. and T. Grundstrum (2002) Calcium regulation of GM-CSF by calmodulin-dependent kinase II phosphorylation of Ets1. Mol. Biol. Cell. 13:4497-4507.

Yordy, J.S. and R.C. Musise-Helmericks (2000) Signal transduction and the Ets family of transcription factors. Oncogene 19:6503-6513.

Cowley, D.O. and B.J. Graves (2000) Phosphorylation represses Ets-1 DNA binding by reinforcing autoinhibition. Genes Dev. 14:366-376.

Rabault, B. and J. Ghysdael (1994) Calcium-induced phosphorylation of ETS1 inhibits its specific DNA binding activity. J. Biol. Chem. 269:28143-28151.

Fleischman, L.F., et al. (1993) c-Ets-1 protein is hyperphosphorylated during mitosis. Oncogene 8:771-780.

Fisher, C.L., et al. (1991) Ligation of membrane Ig leads to calcium-mediated phosphorylation of the proto-oncogene product, Ets-1. J. Immunol. 146:1743-1749.

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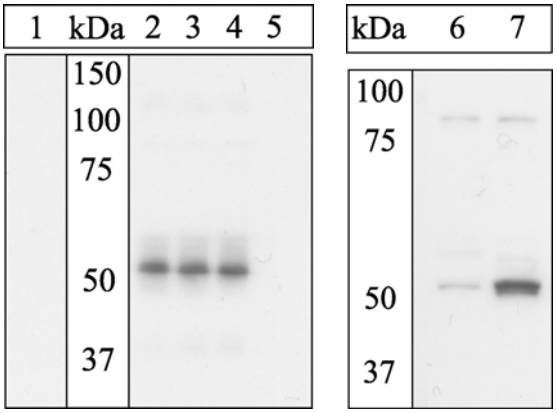

Peptide Competition

Lysates from Jurkat cells either untreated (1, 6), treated with hydrogen peroxide (2-5), or ionophore A23187 (7) were resolved on a 10% polyacrylamide gel and transferred to PVDF. Membranes were blocked with a 3% milk-TBST buffer for one hour at room temperature, then were incubated with the ETS1 [pSpS$^{282/285}$] antibody overnight at 4°C in a 1% milk-TBST buffer, following prior incubation with: no peptide (1-2, 6-7), the non-phosphopeptide corresponding to the immunogen (3), a generic serine containing peptide (4), or the phosphopeptide immunogen (5). After washing, membranes were incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and bands were detected using the Pierce SuperSignal™ method.

The data show that only the peptide corresponding to ETS1 [pSpS$^{282/285}$] blocks the signal, verifying the specificity of the antibody. While complete antibody competition is achieved through preincubation with the dual pSpS$^{282/285}$ phosphopeptide, this antibody is only partially blocked by competition with either the pS^{282} or the pS^{285} phospho-peptides applied singly or together, indicating that the antibody is specifically recognizing a sequence containing the dual phosphorylation site.

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Western Blotting Procedure

1. Lyse approximately 10^7 cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with ddH_2O for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 1% milk and 0.1% Tween 20 overnight at 4°C.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

Cell Lysis Buffer
Formulation:
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM Na_3VO_4
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

Transfer Buffer
Formulation:
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

Tris Buffered Saline
Formulation:
20 mM Tris-HCl, pH 7.4
0.9% NaCl

Blocking Buffer
Formulation:
100 mL Tris buffered saline
3 gm non-fat dried milk
0.1 mL Tween 20

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