S-tag sequence Mouse Monoclonal Antibody

MCA-3H25

Ordering Information
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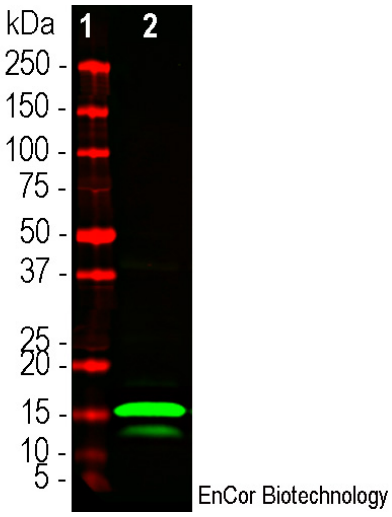
Fax *352-372-7066*

HGNC Name: *NA*
UniProt: *P61823*
RRID: *AB_2923482*
Immunogen: *Peptide derived from the N-terminal sequence of pET30a(+), MHHHHHHSSGLVPRGSGMKETAAAKFERQHMDSPDLGTDDDDKAMADIGSEFC*
Format: *Purified antibody at 1mg/mL in 50% PBS, 50% glycerol plus 5mM NaN_3*
Storage: *Shipped on ice. Store at 4°C for short term, for longer term at -20°C. Avoid freeze / thaw cycles.*
Recommended dilutions:
WB: 1:2,000-1:5,000

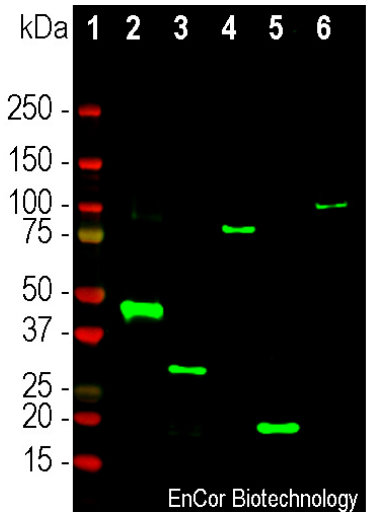
References:

1. Richards FM. On the enzymic activity of subtilisin-modified ribonuclease. PNAS 44:162-6 (1958).
2. Raines RT, McCormick M, Van Oosbree TR, Mierendorf RC. The S.Tag fusion system for protein purification Methods Enzymol. 326:362-76 (2000).

Applications	Host	Isotype	Molecular Wt.	Species Cross-Reactivity
WB	Mouse	IgG1	Not applicable	Co



Western blot analysis of bovine pancreas tissue lysate using mouse mAb to S-tag protein, MCA-3H25, dilution 1:2,000 in green: [1] protein standard (red), [2] 40µg of bovine pancreas. Strong band at about 15 kDa corresponds to intact RNAse A containing the S-tag sequence.



Western blot analysis of 0.25µg of recombinant proteins expressed in pET family vectors and which all contain the S-tag sequence. They were probed with mouse mAb MCA-3H25, dilution 1:5,000 in green: [1] protein standard (red), [2] aldolase A, [2] myelin basic protein, [4] MAP2, P2 projection domain, [5] FOX2 C terminal region and [6] MAP2, P3 projection domain. The antibody binds to the S-tag present in all tested recombinant proteins and reveals protein bands of expected molecular size.

Background:

The story behind the S-tag is quite an interesting one. In the early days of biochemical research it was only feasible to purify and study naturally abundant proteins, so the earliest well characterized proteins were mostly either cytoplasmic enzymes or other abundant proteins such as for example hemoglobin and insulin. In 1958 Frederic M. Richardson showed that an RNAase activity secreted by bovine pancreas could be cleaved by the proteolytic enzyme subtilisin to produce two fragments which he named RNase-S-pep and RNase-S-prot. If the two fragments were separated the RNase activity almost vanished, but activity could be reconstituted if the two components were recombined (1). This interaction was regarded as a model system to study control of enzyme activity, receptor-ligand coupling, allosteric regulation and other forms of biologically important protein-protein interaction. The RNase-S-pep fragment proved to be the N-terminal 20 amino acids of the secreted form of the enzyme while the RNase-S-prot was the C-terminal 104 amino acids. Only the first 15 amino acids of the RNase-s-pep were necessary for the activation of the RNase-S-prot and this peptide became known as the S-tag. The S-tag is one of many intrinsically unstructured peptides which only adopts a defined structure on binding to a structured substrate. The S-tag is incorporated into many vectors including the pET29 and 30 series, pCITE-3 and pCITE-4. The S-tag sequence was incorporated into many expression systems and is detectable with certain antibody reagents allowing researchers to check the size and correct expression of recombinant proteins. Proteins including the S-tag can be purified using a column to which is bound the RNAse-S-prot (2). The modern nomenclature for the enzyme is RNAase A or RNAse 1.

The MCA-3H25 antibody was made against a synthetic 53 amino acid peptide which is the sequence included in pET30a(+) and other vectors, See the "additional info" tab for further information about this peptide. A C-terminal cysteine was added to allow coupling to maleate activated KLH which was used as the immunogen. Numerous clones were screened by their ability to bind the immunogen and then re-screened for inhibition of this binding by the S-tag peptide. The bovine S-tag sequence is not well conserved across species boundaries, so this antibody is not likely to useful as a general marker for RNAase-1 from other species.

Abbreviation Key:

mAb—Monoclonal Antibody pAb—Polyclonal Antibody WB—Western Blot IF—Immunofluorescence ICC—Immunocytochemistry IHC—Immunohistochemistry E—ELISA Hu—Human Mo—Monkey Do—Dog Rt—Rat Ms—Mouse Co—Cow Pi—Pig Ho—Horse Ch—Chicken Dr—D. rerio Dm—D. melanogaster Sm—S. mutans Ce—C. elegans Sc—S. cerevisiae Sa—S. aureus Ec—E. coli.