

FSBA (FSBA 2F5/5): sc-53287

BACKGROUND

Kinases play a major role in many cellular processes by catalyzing the transfer of phosphoryl groups from ATP to a large variety of substrates, including amino acids on target proteins. The reagent 5'-fluorosulfonylbenzoyl-5'-adenosine (FSBA) has been used to identify ATP-binding sites in kinases because it is an ATP-affinity reagent that reacts with nucleophilic amino acids and covalently modifies conserved lysines present in the nucleotide-binding site of kinases. FSBA accomplishes this by first binding to the regulatory site that has a high affinity for ADP and pyrophosphate to increase the V_{max} of the enzyme and then associating with a second regulatory site, a low-affinity site, which increases FSBA's rate of binding. FSBA may subsequently be detected and affinity purified using an anti-FSBA antibody. FSBA also exclusively inactivates the ATP-diphosphohydrolase in human placental mitochondria, thereby inhibiting progesterone synthesis and oxygen consumption.

REFERENCES

1. Feige, J.J., et al. 1983. Identification of the catalytic subunit of an oligomeric casein kinase (G type). Affinity labeling of the nucleotide site using 5'-fluorosulfonylbenzoyl-5'-adenosine. *Biochemistry* 22: 1452-1459.
2. Bullough, D.A. and Allison, W.S. 1986. Three copies of the β subunit must be modified to achieve complete inactivation of the bovine mitochondrial F1-ATPase by 5'-fluorosulfonylbenzoyl-5'-adenosine. *J. Biol. Chem.* 261: 5722-5730.
3. Nishino, T., et al. 1990. Structure of xanthine dehydrogenase from chicken and rat liver: chemical modification of NAD binding site with 5'-FSBA. *Adv. Exp. Med. Biol.* 253B: 173-178.
4. Kim, H.S., et al. 1991. Identification of the ATP binding sites of the carbamyl phosphate synthetase domain of the Syrian hamster multifunctional protein CAD by affinity labeling with 5'-fluorosulfonylbenzoyl-5'-adenosine. *Biochemistry* 30: 10322-10329.
5. Parker, P.J. 1993. Antibodies to fluorylsulfonylbenzoyl-adenosine permit identification of protein kinases. *FEBS Lett.* 334: 347-350.
6. Hartog, A.F., et al. 1997. FSBA modifies both α and β subunits of F1 specifically and can be bound together with AXP at the same α subunit. *Biochim. Biophys. Acta* 1318: 107-122.
7. Oudot, C., et al. 1999. Inactivation of isocitrate dehydrogenase kinase/phosphatase by 5'-fluorosulfonylbenzoyl-5'-adenosine is not due to the labeling of the invariant lysine residue found in the protein kinase family. *Eur. J. Biochem.* 258: 579-585.
8. Flores-Herrera, O., et al. 2002. 5'-fluorosulfonylbenzoyl-5'-adenosine inhibits progesterone synthesis in human placental mitochondria. *Biochim. Biophys. Acta* 1585: 11-18.
9. Renzone, G., et al. 2006. Selective ion tracing and MSn analysis of peptide digests from FSBA-treated kinases for the analysis of protein ATP-binding sites. *J. Proteome Res.* 5: 2019-2024.

STORAGE

Store at 4° C, ****DO NOT FREEZE****. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

SOURCE

FSBA (FSBA 2F5/5) is a mouse monoclonal antibody raised against fluorylsulfonylbenzoyl-adenosine (FSBA, a reactive ATP analog) coupled to carrier proteins.

PRODUCT

Each vial contains 200 μ g IgG in 1.0 ml of PBS with < 0.1% sodium azide and 0.1% gelatin.

FSBA (FSBA 2F5/5) is available conjugated to agarose (sc-53287 AC), 500 μ g/0.25 ml agarose in 1 ml, for IP; to HRP (sc-53287 HRP), 200 μ g/ml, for WB, IHC(P) and ELISA; to either phycoerythrin (sc-53287 PE), fluorescein (sc-53287 FITC), Alexa Fluor® 488 (sc-53287 AF488), Alexa Fluor® 546 (sc-53287 AF546), Alexa Fluor® 594 (sc-53287 AF594) or Alexa Fluor® 647 (sc-53287 AF647), 200 μ g/ml, for WB (RGB), IF, IHC(P) and FCM; and to either Alexa Fluor® 680 (sc-53287 AF680) or Alexa Fluor® 790 (sc-53287 AF790), 200 μ g/ml, for Near-Infrared (NIR) WB, IF and FCM.

Alexa Fluor® is a trademark of Molecular Probes, Inc., Oregon, USA

APPLICATIONS

FSBA (FSBA 2F5/5) is recommended for detection of protein kinases labelled with FSBA by immunoprecipitation [1-2 μ g per 100-500 μ g of total protein (1 ml of cell lysate)] and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500).

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.