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Epidermal Growth Factor, EGF, murine, clone E5 Monoclonal Antibody

Catalog No.: MON 8001-1

Quantity: 1 ml

Specificity
The antibody reacts with mouse EGF in ELISA (10 ng detectable) and in spot blots (1 ng detectable). In immunohistochemistry the antibody reacts with mouse salivary glands.
No crossreaction with rat EGF.

Immunoglobulin type
Murine IgG_1

Use
Detection of murine EGF. In ELISA 10ng is detectable, in spot blots 1ng is detectable.
Use in formalin fixed frozen sections and paraffin sections of salivary glands.

Instructions for use
For use in staining of frozen tissue sections it has to be diluted 1:5-1:25, preferably in phosphate buffered saline. Working dilution for frozen sections is approximately 1:20.

Presentation
1 ml tissue culture supernatant with approximately 20 μg antibody/ml and 1% BSA and 20 mM sodium azide. Catalog No.: MON 8001-1.
5 ml tissue culture supernatant with approximately 20 μg antibody/ml and 1% BSA and 20 mM sodium azide. Catalog No.: MON 8001-5.

Literature
- Beerstecher, H.J., et al., 1988, J. of Histochemistry and Cytochemistry *36*, 1153-1160.

FOR RESEARCH USE ONLY, NOT FOR DRUG, DIAGNOSTIC OR OTHER USE.

Cell Sciences, Inc.
480 Neponset Street
Bldg 12A
Canton, MA 02021

Toll Free: 888-769-1246
Phone: 781-828-0610
Fax: 781-828-0542

E-mail: info@cellsciences.com
Web Site: www.cellsciences.com

Immunoblotting/Spotting protocol

- Homogenize samples in sample buffer containing 50mM Tris-HCL (pH 6.8), 0.01% SDS, 0.6mM glycerol, and 0.33 M ß-mercaptoethanol.

- Heat for 5 min. at 100EC. Cool at room-temperature.

- Centrifugate the samples at 10,000 x g for 5 min.

- Samples of purified mEGF with and without prior heatening in ß-mercaptoethanol were subjected to PAGE according to Maizel.

- After electrophoresis, the gels were soaked for 30 min in H_2O to reduce SDS concentration and then blotted on nitrocellulose paper according to Towbin et al (J. electrophoretic transfer of proteins from polyacrylamide gels to nitro cellulose sheets: procedure and some applications. Proc. Natl Acad. Sci USA 1979;76:4350), with voltage gradient of 5V/cm for periods ranging from 15 min. - 2 hr.

- After electrotransfer of proteins to nitrocellulose paper, the paper was baked overnight at 60EC and the remaining protein binding sites were blocked with 3% ovalbumin in PBS for at least 1 hr.

- Strips of the paper were then incubated with hybridoma culture medium and were developed with RAM-HPO followed by DAB + H_2O.

- Control incubations were done with SP2/0 culture medium.

For analysis of reactions with other proteins containing EGF-like sequences, these proteins were spotted on nitrocellulose strips, which were then allowed to dry: Spots containing such proteins were not baked at 60EC

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