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Product datasheet for AM32087SU-N

Egf Mouse Monoclonal Antibody [Clone ID: E5]

Product data:

Product Type:	Primary Antibodies
Clone Name:	E5
Applications:	ELISA, IHC
Recommended Dilution:	**ELISA.** **Spot Blots.** **Immunohistochemistry on Fixed Frozen Sections:** 1/20. **Immunohistochemistry on Paraffin Sections** of Salivary glands (see **Protocols** for more details.)
Reactivity:	Mouse
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
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.
Formulation:	State: Supernatant State: Tissue Culture Supernatant Stabilizer: 1.0% BSA Preservative: 20 mM Sodium Azide
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	epidermal growth factor
Database Link:	Entrez Gene 13645 Mouse P01132

View online »

Background:

Epidermal growth factor (EGF) has a profound effect on the differentiation of specific cells in vivo and is a potent mitogenic factor for a variety of cultured cells. The EGF precursor is believed to exist as a membrane-bound molecule which is proteolytically cleaved to generate the 53-amino acid peptide hormone that stimulates cells to divide. EGF exerts its actions by binding to the EGFR, a 170 kDa protein.
Epidermal growth factor (EGF) is a key growth factor regulating cell survival. Through its binding to cell surface receptors, EGF activates an extensive network of signal transduction pathways that include activation of the PI3K/AKT, RAS/ERK and JAK/STAT pathways. Because of its key role in driving the proliferation of cells, EGFR is a target of several anti-cancer drugs currently in development.

Synonyms:

Urogastrone, Epidermal growth factor, URG, HOMG4

Note:

Protocol:

Immunoblotting/Spotting

1. Homogenize samples in sample buffer containing 50mM Tris-HCL (pH 6.8), 0.01% SDS, 0.6mM glycerol, and 0.33 M ß-mercaptoethanol.
2. Heat for 5 min. at 100°C. Cool at room-temperature.
3. Centrifugate the samples at 10,000 x g for 5 min.
4. Samples of purified mEGF with and without prior heatening in ß-mercaptoethanol were subjected to PAGE according to Maizel
5. 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.
6. After electrotransfer of proteins to nitrocellulose paper, the paper was baked overnight at 60°C and the remaining protein binding sites were blocked with 3% ovalbumin in PBS for at least 1 hr.
7. Strips of the paper were then incubated with hybridoma culture medium and were developed with RAM-HPO followed by DAB + H_2O.
8. 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 60°C.

Indirect Immunoperoxidase Staining On Frozen Sections

1. 4 to 6 micron thick sections should be used.
2. Sections are thawed, 1-2 hours at room temperature.
3. Tissue is fixed in acetone, 10 minutes.
4. Wash with PBS, 2 x 3 minutes.
5. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
6. Wash with PBS, 3 x 3 minutes.
7. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.

8. Wash with PBS, 3 x 3 minutes.
9. Stain with diaminobenzidin (DAB) solution 10 minutes at room temperature.
10. Wash with running tap water, 3 minutes.
11. Counterstain with Mayer's hematoxylin, 2 minutes.
12. Wash with running tap water, 5 minutes.
13. Dehydrate with increasing solution of ethanol; 50%, 70%, 96%, absolute, 3 minutes each.
14. Clear with xylol, 3 x 3 minutes.
15. Mount with mounting medium (e.g. malinol).

Indirect Immunoperoxidase Staining On Formalin-Fixed And Paraffin Embedded Tissues

1. 4 micron thick sections should be used.
2. Dewax in Xylol, 3 x 3 minutes.
3. Rehydrate in decreasing grades of ethanol:absolute, 96%, 70%, 50%, 3 minutes each.
4. Block endogenous peroxidase activity with freshly made 0.3% H_2O_2 in methanol, 20 minutes.
5. Wash with PBS, 3 x 3 minutes.

Only if trypinsination is required

5a. Incubate sections with 0.1% Trypsin in 0.1% $CaCl_2$ pH 7.6 for 10 minutes at room temperature.
5b. Wash with PBS, 3 x 3 minutes.
6. Cover the sections with 20% normal rabbit serum in PBS or normal human serum and incubate overnight in a humidity chamber at room temperature to reduce non specific background staining.
7. Decant 20% normal rabbit serum.
8. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
9. Wash with PBS, 3 x 3 minutes.
10. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.
11. Wash with PBS, 3 x 3 minutes.
12. Stain with diaminobensidin (DAB) solution, 10 minutes at room temperature. A stock solution of 0.5% DAB in 0.5 DAB in 0.5M Tris/HCl (pH7.4) can be made and stored frozen in the dark. Before use a quantity needed for staining can be thawed and diluted 10x with water. The diluted DAB solution should be filtrated. Just before use H2O2 must be added to a final concentration of 0.01%.
13. Wash with running tap water, 3 minutes.
14. Counterstain with Mayer's hematoxylin, 2 minutes.
15. Wash with running tap water, 2 minutes.
16. Dehydrate with increasing solutions of ethanol:50%, 70%, 96%, absolute, 3 minutes each.
17. Clear with xylol, 3 x 3 minutes.
18. Mount with mounting medium (e.g. malinol).