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# Monoclonal Antibody to EGF - Supernatant

**Alternate names:** Beta urogastrone antibody, Epidermal Growth Factor antibody, Pro epidermal growth factor antibody, URG antibody, Urogastrone antibody

**Catalog No.:** BM2207

**Quantity:** 1 ml

**Concentration:** 20 µg/ml

**Host / Isotype:** Mouse / IgG1

**Clone:** E5

**Format:**
**State:** Liquid supernatant
**Buffer System:** 1% BSA and 20 mM Sodium Azide

**Applications:**
ELISA (10 ng EGF is detectable) and spot blots (1 ng is detectable).
Use in formalin fixed frozen sections (1:20) and paraffin sections of salivary glands. See Protocol for more details.
Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

**Specificity:**
Reacts with EGF. In immunohistochemistry
positive staining is observed in salivary glands.
**Species:** Mouse. Does not work in Rat.
Other species not tested.

**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.
Shelf life: one year from despatch.

**General Readings:**
1. H.J. Beerstecher et al, 1988, J. of Histochemistry and Cytochemistry: vol. 36, 1153-1160.

**Protocols:**
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% H2O2 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% CaCl2 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).