

Product datasheet for **AM32292SU-N**

pan Cytokeratin Mouse Monoclonal Antibody [Clone ID: 80]

Product data:

Product Type:	Primary Antibodies
Clone Name:	80
Applications:	IF, IHC, WB
Recommended Dilution:	Western blotting: Use a preparation of Human callus or keratinocyte as <i>Positive Control</i> . Immunofluorescence. Immunohistochemistry on Frozen Sections: ~1/50. Immunohistochemistry on Paraffin Embedded Tissue: ~1/10 (Dilution buffer PBS with 1% BSA). To obtain a positive result in paraffin embedded tissue a TUF pretreatment is recommended. See Protocols for more details.
Reactivity:	Human, Mouse, Rat
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	Callus cytokeratins isolated from fresh Human skin tissue.
Specificity:	This antibody clone <i>80</i> recognizes most Human keratins in Immunoblots. The antibody stains in Immunohistochemistry and Immunofluorescence tests all types of keratin containing (that is epithelial) cells in Frozen Sections of various tissues, with the exception of myoepithelial cells. It also stains Mouse and Rat keratin. No cross-reactivity in Neurofilament, Vimentin, GFAP, and Desmin.
Formulation:	State: Supernatant State: Liquid Tissue Culture Supernatant Preservative: 10mM Sodium Azide, 1% FCS
Concentration:	lot specific
Conjugation:	Unconjugated
Storage:	Store the antibody undiluted at 2-8°C.
Stability:	Shelf life: one year from despatch



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Background: Cytokeratin is a family of basic and acidic proteins, present in dermal tissues. Each cytokeratin is formed by heterotetramers of different types of keratin, that span from 1 to 18. In keratinized epidermis, 50 kD keratin is present in the basal layer, while 56.5 kD keratin is present in suprabasal layers, where as 58 kD keratin is present in the basal and suprabasal layers, while 65 to 67 kD keratin is present in the cells above the basal layers.

Synonyms: pan Keratin, Cytokeratin pan-reactive

Note: Protocol: **Indirect Immunoperoxidase Staining On Frozen Sections (DAB):**

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 Frozen sections (AEC):

1. Frozen sections should have been fixed in acetone for 10 min.
 2. Incubation in antisera 40-60 min.
 3. Incubation in conjugate (e.g. peroxidase conjugated anti mouse IgG), 30 min.
 4. Wash in PBS 2 x 5 min.
 5. Incubation in AEC, 0.0.1% H₂O₂, 10 min.
- Preparation substrate: a) 5 mg AEC (3-amino-9-ethylcarbazole) is solubilized in 0.5 ml DMF (dimethylformamide). A glass (or acetone resistant plastic) tube or pipet should be used! b) add 9.5 ml. 0.05M NaAc buffer, pH 4.9. c) add 5 ul 30% H₂O₂.
6. Wash in demi water, 2 x 5 min.
 7. Slightly counterstain in hematoxylin e.g. 10 sec.
 8. Wash in tap water until sections are blue.
 9. Mount in aquamont and examine by microscopy.