

Product Datasheet

TOR/mTOR Antibody (Y391)

NB110-56996

Unit Size: 0.1 ml

Store at -20C. Avoid freeze-thaw cycles.

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Product Information	
Unit Size	0.1 ml
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	Y391
Preservative	0.01% Sodium Azide
Isotype	IgG
Purity	Protein A or G purified
Buffer	49% PBS, 0.05% BSA and 50% Glycerol
Product Description	
Host	Rabbit
Gene ID	2475
Gene Symbol	MTOR
Species	Human, Mouse, Rat
Reactivity Notes	Human, Mouse, Rat. For Mouse and Rat this antibody is recommended for Western Blot only.
Immunogen	A synthetic peptide corresponding to residues near the C-term of PI3K/PI4K domain of human mTOR/FRAP was used as an immunogen
Notes	Produced using Abcam's RabMab® technology. RabMab® technology is covered by the following U.S. Patents, No. 5,675,063 and/or 7,429,487.
Product Application Details	
Applications	Western Blot, Flow Cytometry, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Immunoprecipitation
Recommended Dilutions	Western Blot 1:1000-2000, Flow Cytometry 1:80, Immunohistochemistry, Immunocytochemistry/ Immunofluorescence 1:50-100, Immunoprecipitation 1:50, Immunohistochemistry-Paraffin 1:250-500
Application Notes	This product is useful for: Western Blot, Immunohistochemistry-Paraffin, Immunocytochemistry, Immunoprecipitation, Flow Cytometry. In Western blot this antibody detects a band at approximately 289kDa.

Publications

Marioni G, Ottaviano G, Lovato A et al. Expression of maspin tumor suppressor and mTOR in laryngeal carcinoma Am J Otolaryngol [PMID: 31732312] (IHC-P, IF/IHC, Human)

Lionello M, Lovato A, Staffieri A et al. The EGFR-mTOR pathway and laryngeal cancer angiogenesis. Eur Arch Otorhinolaryngol. 2013-09-25 [PMID: 24065188] (IHC-P, Human)

Xiao L, Wang YC, Li WS et al. The role of mTOR and phospho-p70S6K in pathogenesis and progression of gastric carcinomas: an immunohistochemical study on tissue microarray. J Exp Clin Cancer Res 2009-12-01 [PMID: 20003385]

Marioni G, Staffieri A, Lora L et al. mTOR expression and prognosis in elderly patients with laryngeal carcinoma: Uni- and multivariate analyses. Oral Oncology. 2012-01-27 [PMID: 22285859]



Procedures

Protocol specific for mTOR Antibody (NB110-56996)

Immunohistochemistry Protocol for Paraffin-embedded Tissues

1. Solutions and reagents

1.1. Xylene

1.2. Ethanol, anhydrous denatured, histological grade (100%, 95%, 70%)

1.3. Washing buffer: TBST washing buffer: 1XTBS/0.1% Tween-20

To prepare stock solution of 10X TBS: add 24.2 g Trizma base and 80 g sodium chloride to 1L of dH₂O. Adjust pH to 7.6.

Working solution. 1XTBST/0.1% Tween-20: add 100ml 10XTBS to 900 ml dH₂O. Add 1 ml Tween-20 and mix well.

1.4. Distilled water (dH₂O)

1.5. Antigen Retrieval Solution:

0.01M Sodium Citrate Buffer, pH 6.0

To prepare stock solutions:

Solution A. 0.1 M citric acid solution: dissolve 21.0 g of citric acid, monohydrate (C₆H₈O₇.H₂O) in 1 liter of dH₂O.

Solution B. 0.1M sodium citrate solution: dissolve 29.4 g trisodium citrate dihydrate (C₆H₅Na₃O₇.2H₂O) in 1 liter of dH₂O.

Working solution: Add 9 ml of Stock solution A and 41 ml of stock solution B to 450 ml of dH₂O. Adjust pH to 6.0.

1.6. 3% Hydrogene Peroxide

1.7. Blocking buffer: PBS (Dulbeccos Phosphate Buffered Salts, 1X, catalog #21-031-CV from Mediatech, Inc.) + 10% serum (serum origin depends on the host of the secondary antibody)

1.8. Hematoxylin QS (catalog #H-3404 from Vector Laboratories, Inc.)

1.9. Permanent Mounting medium (VectaMount, catalog# H-5000 Vector Laboratories, Inc.)

2. Protocol

2.1. Deparaffinization/Rehydration

2.1.1. Heat slides in an oven at 65C for 1 hour.

2.1.2. De-paraffinize/hydrate using the following series of washes: two Xylene washes (5 min each), followed by two 100% ethanol rinses (5 min each), followed by 95% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, followed by H₂O and a TBST wash for 5 min on a shaker.

2.2. Antigen Retrieval

2.2.1. Immerse slides into staining dish containing Antigen Retrieval Solution.

2.2.2. Place covered staining dish into the rice cooker. Add 120 ml of dH₂O.

2.2.3. When cook is turned to warm (about 20 to 30 min), unplug the cooker and remove the staining dish to the bench top.

2.2.4. Allow to cool down, without cover, for 20 min.

2.3. Staining

2.3.1. Wash slides with TBST for 5 min on a shaker.

2.3.2. Inactivate endogenous peroxidase by covering tissue with 3% hydrogen peroxide for 10 min.

2.3.3. Wash slides three times with TBST (3 min each on a shaker).

2.3.4. Block slides with the blocking solution for 1 hour.

2.3.5. Dilute primary antibody in the blocking buffer per recommendation on the data sheet.

2.3.6. Apply primary antibody to each section and incubate overnight in the humidified chamber (4C).

2.3.7. Wash slides three times with TBST (3 min each on a shaker).

2.3.8. Apply to each section secondary HRP-conjugated anti-rabbit antibody diluted in the blocking solution per manufacturers recommendation; incubate for 1 hour at room temperature.

2.3.9. Wash slides three times with TBST (3 min each on a shaker).

2.3.10. Add freshly prepared DAB substrate to the sections.

2.3.11. Incubate tissue sections with the substrate at room temperature until suitable staining develops (generally 2 to 5 min).

2.3.12. Rinse sections with water.

2.3.13. Counterstain with Hematoxylin.

2.3.14. Rinse sections with water.

2.3.15. Dehydrate samples using two rinses with 100% Ethanol (20 dips per rinse) followed by two rinses with Xylene (30 dips per rinse).

2.3.16. Mount coverslips on slides using Permount medium.



Novus Biologicals USA

10730 E. Briarwood Avenue
Centennial, CO 80112
USA
Phone: 303.730.1950
Toll Free: 1.888.506.6887
Fax: 303.730.1966
nb-customerservice@bio-techne.com

Bio-Techne Canada

21 Canmotor Ave
Toronto, ON M8Z 4E6
Canada
Phone: 905.827.6400
Toll Free: 855.668.8722
Fax: 905.827.6402
canada.inquires@bio-techne.com

Bio-Techne Ltd

19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB, United Kingdom
Phone: (44) (0) 1235 529449
Free Phone: 0800 37 34 15
Fax: (44) (0) 1235 533420
info.EMEA@bio-techne.com

General Contact Information

www.novusbio.com
Technical Support: nb-technical@bio-techne.com
Orders: nb-customerservice@bio-techne.com
General: novus@novusbio.com

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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