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Product Information

Anti-mTOR (FRAP)

Developed in Rabbit, Affinity Isolated Antibody

Product Number **T 2949**

Product Description

Anti-mTOR (mammalian target of Rapamycin) (FRAP) is developed in rabbit using a synthetic peptide corresponding to amino acid residues 2433-2450 of human mTOR with N-terminal added cysteine, conjugated to KLH as immunogen. The corresponding sequence is identical in mouse and differs by one amino acid in rat. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-mTOR (FRAP) specifically recognizes human, mouse and rat mTOR (289 kDa). Applications include immunoblotting, immunoprecipitation and immunofluorescence.

mTOR, also named FRAP/RAFT/RAPT/SEP, is a serine/threonine protein kinase, an evolutionary conserved large member of the phosphoinositol kinase-related kinase (PIKK) family that also includes DNA-PK, ATM, ATR and several other proteins.¹⁻⁵ mTOR is involved in the regulation of cell growth through activation of translation initiation in response to nutrients such as amino acids (mainly leucine), growth factors, insulin, and mitogens. mTOR activates the translational machinery by activating the ribosomal p70^{S6k} protein kinase (S6K1) and by inhibiting the eIF4E inhibitor 4E-BP1. mTOR is thought to be involved in numerous additional cellular functions and intracellular events that coordinate growth and proliferation, actin organization, membrane traffic, secretion, protein degradation, protein kinase C signaling, ribosome biogenesis and t-RNA synthesis.¹

Rapamycin, a lipophilic bacterial macrolide fungicide, can complex with the immunophilin FK-506 binding protein FKBP12 peptide prolyl cis/trans isomerase. Interaction of this FKBP12-rapamycin complex with mTOR inhibits its function. The immunosuppressive effect of rapamycin is due, in part, to its ability to interfere with T-cell activation at the level of protein translation. Interference at this level also appears to underlie the suppression of neoplastic cell growth by

Rapamycin and its analogs.¹⁻⁵ It is suggested that mTOR may sense cellular ATP levels and suppress protein synthesis when ATP levels decrease.⁶ mTOR is phosphorylated at serine 2448 via the PI3 kinase/AKT pathway and is autophosphorylated at serine 2481.⁷⁻⁸ mTOR is located in the cytoplasm, in association with mitochondria, and in the nucleus depending on the cell type.⁹⁻¹¹

Reagent

Anti-mTOR is supplied at approximately 1 to 1.5 mg/ml as a solution in 0.01 M phosphate buffered saline pH 7.4 containing 1% BSA and 15 mM sodium azide as preservative.

Precautions and Disclaimer

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

Store at -20 °C. For extended storage, upon initial thawing, freeze in working aliquots. Do not store in frost-free freezers. Avoid repeated freezing and thawing to prevent denaturing the antibody. Working dilution samples should be discarded if not used within 12 hours. The antibody is stable for at least 6 months when stored appropriately.

Product Profile

A minimum working dilution is determined by immunoblotting using chemiluminescent detection reagent. The 1:2,000 dilution is recommended for whole extracts of rat brain tissue, and 1:500 for whole extracts of differentiated mouse C2C12 skeletal muscle cells. Additional weak bands may be detected when immunoblotting some tissue extracts. 1.0 to 1.5 µg of the antibody immunoprecipitates mTOR from 300 µg RIPA extract of human transformed kidney HEK 293T cells.

Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working concentration by titration test.

References

1. Schmelzle, T. and Hall, M.N., *Cell*, **103**, 253-262 (2000).
2. Brown, E.J., et al., *Nature*, **369**, 756-758 (1994).
3. Sabatini, D.M, et al., *Cell*, **78**, 35-43 (1994).
4. Chiu, M. I., and Berlin, V., *Proc. Natl. Acad. Sci., USA*, **91**, 12574-12578 (1994).
5. Sabers, C.J., et al., *J. Biol. Chem.*, **270**, 815-822 (1995).
6. Dennis, P.B., et al., *Science*, **294**, 1102-1105 (2001).
7. Nave, B.T., et al., *Biochem. J.*, **344**, 427-431 (1999).
8. Peterson, R.T., et al., *J. Biol. Chem.*, **275**, 7416-7423 (2000).
9. Kim, J.E. and Chen, J., *Proc. Natl. Acad. Sci. USA*, **97**, 14340-14345 (2000).
10. Desai, B.N., et al., *Proc. Natl. Acad. Sci. USA*, **99**, 4319-4324 (2002).
11. Zhang, X., et al., *J. Biol Chem.*, **277**, 28127-28134 (2002).

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