

Everolimus

✓ 1 mg

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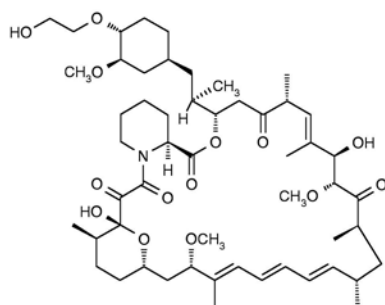
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For Research Use Only. Not For Use In Diagnostic Procedures.

Background: Everolimus, also known as RAD001, is an immunosuppressant analog of rapamycin. Everolimus forms a complex with FKBP12 with an $IC_{50} = \sim 2$ nM in FK506 competitive binding assays (1,2), and this complex then binds to and inhibits mTORC1 (3). Studies have shown that everolimus treatment of cells can lead to the dephosphorylation of mTOR downstream targets (4-7), inhibition of VEGF- and bFRF-stimulated proliferation in HUVE cells (6), and reduction of hypoxia-induced HIF-1 protein levels (7).

Molecular Formula: $C_{53}H_{83}NO_{14}$



Molecular Weight: 958.22 g/mol

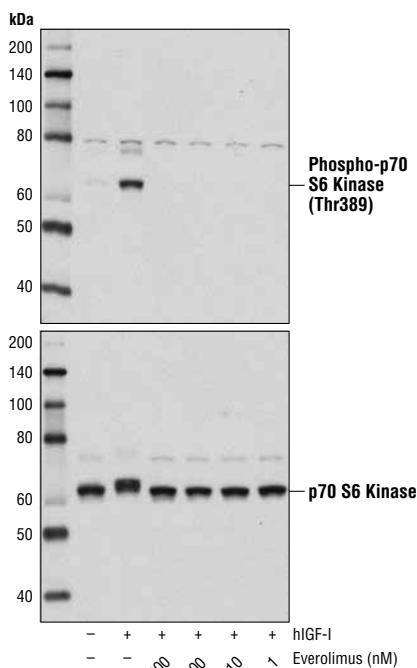
Solubility: Soluble in DMSO or ethanol at 100 mg/ml; poorly soluble in water with maximum solubility ~ 1 -10 μ M.

Purity: >99%

Directions for Use: Everolimus is supplied as a lyophilized powder. For a 1 mM stock, reconstitute the 1 mg in 1.04 ml DMSO. Working concentrations and length of treatment can vary depending on the desired effect, but it is typically used at 10-100 nM either as a pretreatment for 0.5-1 hr prior to treating with a stimulator or is used alone with varying treatment times lasting up to 24 hr.

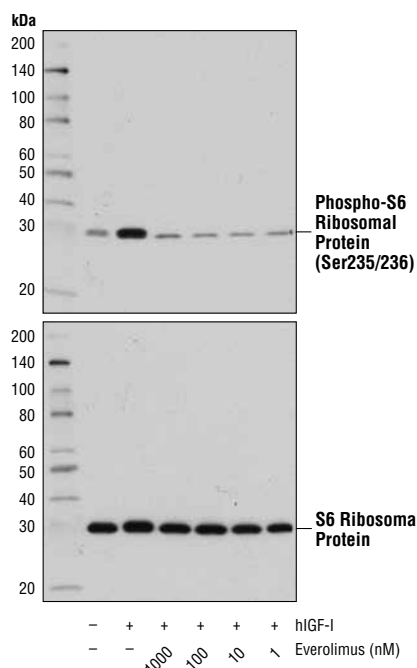
Background References:

- (1) Sedrani, R. et al. (1998) *Transplant Proc* 30, 2192-4.
- (2) Schuler, W. et al. (1997) *Transplantation* 64, 36-42.
- (3) Houghton, P.J. (2010) *Clin Cancer Res* 16, 1368-72.
- (4) Rosich, L. et al. (2012) *Clin Cancer Res* 18, 5278-89.
- (5) Cao, C. et al. (2006) *Cancer Res* 66, 10040-7.
- (6) Lane, H.A. et al. (2009) *Clin Cancer Res* 15, 1612-22.
- (7) Knaup, K.X. et al. (2009) *Mol Cancer Res* 7, 88-98.



Storage: Store lyophilized or in solution at -20°C , desiccated. Protect from light. In lyophilized form, the chemical is stable for 24 months. Once in solution, use within 3 months to prevent loss of potency. Aliquot to avoid multiple freeze/thaw cycles.

◀ Western blot analysis of extracts from HeLa cells, serum-starved overnight and treated with hIGF-I #8917 (100 ng/ml, 10 min) either with or without Everolimus pretreatment (1 hr) at the indicated concentrations, using Phospho-p70 S6 Kinase (Thr389) Antibody #9205 (upper) or p70 S6 Kinase (49D7) Rabbit mAb #2708 (lower).



◀ Western blot analysis of extracts from HeLa cells, serum-starved overnight and treated with hIGF-I #8917 (100 ng/ml, 10 min) either with or without Everolimus pretreatment (1 hr) at the indicated concentrations, using Phospho-S6 Ribosomal Protein (Ser235/236) (D57.2.2E) XP® Rabbit mAb #4858 (upper) or S6 Ribosomal Protein (5G10) Rabbit mAb #2217 (lower).

Applications Key: W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide
Species Cross-Reactivity Key: H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine
 Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—horse All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.