

Characterization of the HTRF assay for a truncated mammalian target of rapamycin(mTOR) kinase

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The mammalian target of rapamycin (mTOR) is a serine/threonine kinase that belongs to the phosphoinositide 3-kinase (PI3K)-related kinase family (PIKK) and regulates proliferative, metabolic and survival pathways. The regulation of proliferation and metabolism occurs at the translation initiation level through two distinct pathways. The mTORC1 complex consists of mTOR bound to a scaffolding protein called raptor which modulates the binding of kinase substrates. Raptor associated mTOR is the target of the Rapamycin / FKBP12 complex. TORC2 binds a different scaffolding protein called rictor which was implicated in cytoskeleton reorganization and was recently identified as the kinase complex (PDK2) responsible for phosphorylating the C-terminal site of AKT-1, Ser473.

We have developed a high throughput HTRF assay to identify a direct kinase inhibitor of mTOR. This type of compound would inhibit both cell growth and proliferation due to the inhibition of the mTOR translational pathway and would inhibit the cell survival activity induced by the TORC2 complex activation of AKT. The latter activity would potentially lead to increased tumor cell inhibition and distinguish the proposed inhibitor from rapamycin and its analogues.

The assay is performed in 384 low volume plates at 30°C or room temperature. The kinase action was initiated by adding GST-S6K and ATP in the well with mTOR and testing compounds. The detection buffer, consisting of Anti-phospho p70S6K and Anti-GST XL665/GSTS6K, catch the phosphorylated substrate. The fluorescence is measured at 620 nm (Cryptate) and 665 nm (XL665) on the Rubystar which calculates a ratio (665/620) for each well.

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EDUCATION

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PROFESSIONAL EXPERIENCE

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Research Scientist, February, 1986 to May, 1988.
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Research Associate, September, 1982 to February, 1986.
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PERSONAL INFORMATION

Born, December 13, 1951 in Korea
Naturalized Citizen of the United States of America (1980)
Married, with two sons
Serve as an Elder in Praise Presbyterian Church in Somerset, NJ