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PRODUCT DATA SHEET

Product Name: Staurosporine
Catalog Number: 6212
Size: 1 mg/vial
Storage Temperature: -20°C

Product Description
Molecular Formula: $C_{28}H_{26}N_4O_3$
Molecular Weight: 466.5
CAS Number: 62996-74-1

Staurosporine is a natural product derived alkaloid which is isolated from *Streptomyces staurospores* cultures. This protein kinase inhibitor belongs to a family of kinase inhibitors containing an indole carbozol chromophore (Reviewed in Hidaka and Kobayashi 1). Staurosporine is a potent inhibitor of a number of kinases including: Protein kinase C (PKC), cAMP dependent protein kinase- protein kinase A (PKA), tyrosine protein kinase, phosphorylase kinase, and Ca^2+/calmodulan-dependent protein kinase (1-6). The ATP binding site on these kinase enzymes appears to be targeted by these *Streptomyces* derived inhibitors (1). Experiments using [^{3}H]- staurosporine inhibition of purified PKC, PKA, tyrosine protein kinase and Ca^2+/calmodulan-dependent protein kinase enzymes revealed dissociation constants (K_D) ranging from 2-10 nM (2, 4). Human promyelocytic leukemia (HL-60) cells were found to be sensitive to apoptosis induction from a 2 hour exposure to staurosporine concentrations as low as 0.1 μM (7).

Inhibition of these intracellular kinases by staurosporine leads to the induction of apoptosis as exhibited by the classic chromatin condensation leading to the formation of micronuclear bodies and reduced cell volumes. DNA is subsequently cleaved into oligonucleosomal fragments (laddering) as evidenced by agarose gel analysis (7, 8). Staurosporine was determined to be an effective apoptosis inducing agent in virtually all cell lines that were exposed to this kinase inhibiting agent (7, 8).

Brief Protocol

1. Dissolve staurosporine powder in tissue culture grade DMSO to obtain a 1 mM staurosporine concentration.
   a. Example calculation:
   Staurosporine MW = 466.5
   1 mM Staurosporine = 1 x 466.5 μg/mL or 0.4665 mg/mL
   To reconstitute a 1 mg vial of staurosporine:
   1 mg/0.4665mg/mL = 2.14 mL DMSO reconstitution volume to obtain a 1 mM staurosporine stock solution.

2. Prepare 50 – 100 uL aliquots of the DMSO solubilized staurosporine stock solution and store them frozen at < -20° C. A frozen vial of staurosporine may only be rethawed 2X before it must be discarded. Vials which have been thawed 1X should be marked to indicate this so that they go through only 1 more freeze-thaw before being discarded.
3. Spike cell cultures at a staurosporine concentration of 1 μM in the cell culture media. This equates to a 1 μL spike of the 1 mM staurosporine stock per mL of the cell culture suspension. This concentration works well for inducing many cell lines including HL-60 and Jurkat cells when using a 4-5 hour 37° C incubation period. Typical cell suspension concentrations that have been used for this staurosporine induction protocol range from 1 x 10^5 – 1 x 10^6 cells/mL.

4. Perform time course studies on your particular cell line to ascertain the optimal staurosporine concentration and exposure time required to achieve good apoptosis induction levels in your experimental system.
5. Proceed with your experimental apoptosis induction model system.

References

1. Hidaka, H. and Kobayashi, R. 1992. Pharmacology of protein kinase inhibitors. *Annu. Rev. Pharmacol. Toxicol*. 32: 377-397.
2. Tamaoki, T. et al. 1986. Staurosporine, a potent inhibitor of phospholipids/Ca++ dependent protein kinase. *Biochem. Biophys. Res. Commun*. 135: 397-402.
3. Matsumoto, H. and Sasaki, Y. 1989. Staurosporine, a protein kinase C inhibitor interferes with proliferation of arterial smooth muscle cells. *Biochem. Biophys. Res. Commun*. 158: 105-109.
4. Herbert, J. et al. 1990. Characterization of specific binding sites for [^{3}H]-staurosporine on various protein kinases. *Biochem. Biophys. Res. Commun*. 171: 189-195.
5. Elliott, L. et al. 1990. K252a is a potent and selective inhibitor of phosphorylase kinase. *Biochem. Biophys. Res. Commun*. 171: 148-154.
6. Fallon, R. 1990. Staurosporine inhibits a tyrosine kinase in human hepatoma cell membranes. *Biochem. Biophys. Res. Commun*. 170: 1191-1196.
7. Bertrand, R. et al. 1994. Induction of a common pathway of apoptosis by staurosporine. *Exp. Cell Res*. 211: 314-321.
8. Boix, J. et al. 1997. Characterization of the cell death process induced by staurosporine in human neuroblastoma cell lines. *Neuropharmacology* 36: 811-821.