

Staurosporine

From *Streptomyces* species lyophilized

Cat. No. 11 055 682 001

500 µg

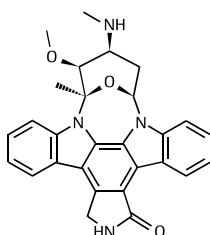
Version 07

Content version: December 2005

Store at +2 to +8° C

Product overview

Formulation	Lyophilizate
Toxicity	LD ₅₀ = 6.6 mg/kg (i.p. in mice)
Structure	



Characteristics

Formula	C ₂₈ H ₂₆ N ₄ O ₃
Molecular weight	466.5
Appearance	pale yellow powder

Biochemical properties and applications

Staurosporine, a microbial alkaloid antibiotic found by Omura, S. *et al.*, has been reported to have antifungal and strong hypotensive effects (1). It also seems that staurosporine is the most potent inhibitor of the phospholipid/Ca²⁺-dependent protein kinase C with an IC₅₀ of 2.7 nM regarding the enzyme from rat brain (2).

Compared to other known protein kinase C inhibitors like trifluoperazine, chloropronaline and polymyxin B staurosporine is about 3 orders of magnitude more effective. This potent inhibitory effect makes the compound very valuable in investigating the role of protein phosphorylation by protein kinase C, Ca²⁺ mobilization by inositol phosphates and provides a useful tool for the isolation and purification of protein kinase C. It also inhibits cAMP- and cGMP-dependent protein kinase with K_i-values around 7 nM and the protein tyrosine kinase activity of p60^{v-src} with an IC₅₀ value of 6.4 nM (3). Thus, Staurosporine is a very potent inhibitor of protein tyrosin kinases as well.

Besides direct blocking of protein kinases staurosporine exhibits strong cytotoxic effects on various tumor cell lines (2) and inhibits platelet aggregation (4, 5) with an IC₅₀ of 3.4 µM or 11.6 µM, depending on induction of aggregation induced by either collagen or ADP, respectively.

The compound does not interfere with the binding of phosphatidylserine and phorbol esters to protein kinase C suggesting different binding sites.

Additionally, staurosporine does not compete with Ca²⁺, histones, DAG and ATP (2).

Different authors show correlation of protein kinase inhibition by staurosporine and apoptosis induction (10, 11,12,13).

Inhibition of protein kinases by staurosporine

The mechanism of the inhibition of protein kinases by staurosporine is unclear so far.

Nakano, H. *et al.* (3) state that staurosporine may bind to the catalytic domain of serine/threonine- as well as tyrosine-kinases. In the presence of ATP, 100 µM even low staurosporine concentrations (1-8 nM) were capable of inhibiting p60^{v-src} phosphorylation ruling out a competition of both compounds. As a consequence staurosporine should also inhibit phosphorylation *in vivo* in the presence of physiological ATP concentrations.

Herbert, J. M. *et al.* (9) showed ATP compete with [³H]-staurosporine binding to PKC with an IC₅₀ of ATP, 500 nM in the presence of tritiated inhibitor, 2 nM. The same authors described that isoquinolinesulfonamides (H-7 and H-8) and naphthalenesulfonamides (*e.g.* W-7), classes of non-selective protein kinase inhibitors, failed to antagonize [³H]-staurosporine binding to different protein kinases therefore suggesting distinct binding sites.

The staurosporine concentration for half maximal inhibition of PKC in presence of ATP, 10 µM and histone H1, 10 µM has been determined to be 10⁻⁸ M (see fig. 1). In order to block the PKC totally *in vitro* a staurosporine concentration of 10⁻⁷ M is required.

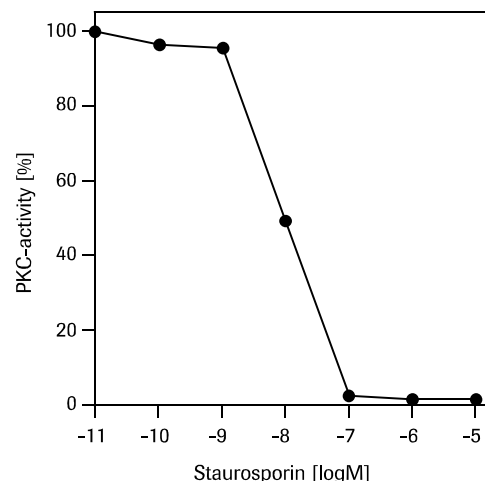


Fig. 1: Inhibition of protein kinase C by staurosporine.

Solubility

Staurosporine is soluble in DMSO (dimethylsulfoxide), DMF (dimethylformamide) (both 20 mg/ml) and pyridine, slightly soluble in chloroform, methanol, acetone and insoluble in water and non-polar organic solvents.

Reconstitution

The powder in the vial may be reconstituted in 500 µl DMSO.

Working concentration

10 nM to 10 µM

Identification

The UV spectrum shows λ_{max} at 243, 292 (highest absorption), 335, 356, and 372 nm in methanol [ε_{292nm} = 57.38 (l × mmol⁻¹ × cm⁻¹)]

Quality control	Purity > 98% checked by absorbance at 292 nm. Chromatographically homogeneous.
Storage/Stability	The powder is stable at +2 to +8°C, stored dry and protected from light until the expiration date printed on the label. Note: Dissolved in DMSO (1 mg/ml) staurosporine is stable for at least 3 months at +2 to +8°C. It can also be stored in aliquots at -15 to -25°C.

Assay conditions and determination of activity

General Protein kinase C, like other serine/threonine-kinases, transfers the γ -phosphate group from ATP to serine or threonine residues of target proteins or peptides. For the enzymatic determination of protein kinase C synthetic substrate peptides as well as proteins may be used as phosphate acceptors (s. table 1). Depending on the type of acceptor different assay procedures have to be followed.

Table 1: Substrates of protein kinase C (PKC)

Substrate	k_m -value [μ M]	V_{max} [μ mol/min \times mg]	References
Histone H1*	0.6	0.83	(6)
[Ser ²⁵]-PKC (19-31)	0.2	8.00	(7)

Additional reagents required The experimental set up for activated PKC has a final volume of 200 μ l consisting of final concentrations of the following reagents:

- Tris*-HCl; 20 mM, pH 7.5;
- MgCl₂; 10 mM
- CaCl₂; 200 μ M;
- Phosphatidylserine¹⁾; 8 μ g/ml,
- Diolein¹⁾; 0.8 μ g/ml;
- Phosphateacceptor²⁾; 10 μ M;
- Protein kinase C, 0.05 U (1 unit corresponds to the transfer of 1 mmole phosphate per min to the acceptor at acceptor saturation)
- [³²P]ATP, 10 μ M (50–100 cpm/pmol)²⁾.

¹⁾ The lipids (solubilized in chloroform) are mixed, the solvent is removed by aeration with nitrogen and the oily residual is brought into solution with 10 mM CHAPS*)

²⁾ In order to guarantee substrate saturation and linearity of the reaction not more than 10% of the acceptor should be phosphorylated at the end of the reaction.

Determination of basal PKC The determination of the basal PKC activity is achieved by replacing the stimulating agents phosphatidylserine and diolein by H₂O whereas CaCl₂, 200 μ M is replaced by EGTA*, 500 μ M.

Protocol Procedure using histone as substrate (8).

Step	Action
1	The kinase reaction is started by addition of either: <ul style="list-style-type: none"> • histone H1, • ATP or • enzyme
2	Incubate 3 min at +30°C.
3	Termination of the reaction: Add 1 ml TCA (trichloroacetic acid, 25%), and 0.1 ml bovine serum albumin (BSA)*, 1% Note: BSA is added as a carrier to ensure a quantitative precipitation of the substrate.
4	Incubate 5 min at 0°C.
5	Filter samples by suction through glasfiber filters.
6	Wash with ice-cold 10% TCA.
7	Determination of the TCA-precipitated radioactivity (phosphorylated histone H1) by <ul style="list-style-type: none"> • liquid scintillation counting or • measuring the Cerenkov-scintillation (³H-channel).

Procedure using basic substrate peptides (7) The composition and assay condition are identical as above mentioned. However, using [Ser²⁵]-PKC (19-31) as the substrate the termination of the reaction is accomplished by spotting aliquots on phosphocellulose filters. Not-incorporated radioactivity is separated by washing with 75 mM phosphoric acid. The radioactivity is determined again by liquid scintillation or by measuring the Cerenkov-scintillation (³H-channel).

References

- 1 Omura, S. et al. (1977) *J. Antibiot.* **30**, 275–281.
- 2 Tamaoki, T. et al. (1986) *Biochem. Biophys. Res. Comm.* **135**, 397–402.
- 3 Nakano, H. et al. (1987) *J. Antibiot.* **40**, 706–708.
- 4 Oka, S. et al. (1986) *Agric. Biol. Chem.* **50** (11), 2723–2727.
- 5 Schächtele, C. et al. (1988) *Biochem. Biophys. Res. Comm.* **151**, 542–547.
- 6 Wise, B. C. et al. (1982) *J. Biol. Chem.* **257**, 8489–8495.
- 7 House, H. & Kemp, B. E. (1987) *Science* **238**, 1726–1728.
- 8 Kitano, T. et al. (1986) *Meth. Enzym.* **124**, 349–352.
- 9 Herbert, J. M. (1990) *Biochem. Biophys. Res. Comm.* **171**, 189–195.
- 10 Bertrand, R. et al. (1994) *Exp. Cell Res.* **211**, 314–321;
- 11 Couldwell, W.T. et al. (1994) *FEBS Lett.* **345**, 43–46;
- 12 Jarvis, W.D. et al. (1994) *Cancer Res.* **54**, 1707–1714;
- 13 Lucas, M. et al. (1994) *Biochem. Pharmacol.* **47**, 667–672;

Changes to previous version Regulatory Disclaimer updated

Trademarks All product names and trademarks are the property of their respective owners.

Regulatory Disclaimer For life science research only. Not for use in diagnostic procedures.

* available from Roche Applied Science

Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site** at:

www.roche-applied-science.com/support

To call, write, fax, or email us, visit the Roche Applied Science home page, www.roche-applied-science.com, and select your home country. Country-specific contact information will be displayed. Use the Product Search function to find Pack Inserts and Material Safety Data Sheets.



Roche Diagnostics GmbH
Roche Applied Science
68298 Mannheim
Germany