

---

**Product Data Sheet**

---

Product Name: A 419259

Cat. No.: GC35209

**Chemical Properties**

Cas. No. 364042-47-7

SMILES NC1=C2C(N([C@H]3CC[C@H](N4CCN(C)CC4)CC3)C=C2C5=CC=C(OC6=CC=CC=C6)C=C5)=NC=N1Formula  $C_{29}H_{34}N_6O$  M.Wt 482.62

Solubility 55 mg/mL in water Storage Store at -20°C

General tips For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Condition Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.

Structure **Protocol**

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

---

## Product Data Sheet

---

### **Kinase experiment:**

In vitro kinase assays are performed on His(6)-tagged Lck (residues 62-509) and full-length c-Abl purified from Sf-9 cells, and commercial sources of Lyn, Src and PKC. Lck, Lyn, Src and Abl activities are measured with an ELISA-based assay. Flat bottom 96-well ELISA plates are incubated with a 200 µg/mL solution of Poly(Glu,Tyr) 4 : 1 substrate in phosphate buffered saline (PBS) for 1 h at 37°C and then washed with PBS containing 0.1% Tween-20 (PBS-T). Inhibitor dilutions are added to the washed plates already containing the appropriate enzyme in kinase assay buffer (250 mM Mopso, pH 6.75, 10 mM MgCl<sub>2</sub>, 2 mM MnCl<sub>2</sub>, 2.5 mM DTT, 0.02% BSA, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 5% DMSO, 5 µM ATP). After incubation at room temperature for 20 min, plates are washed three times with PBS-T and plate-bound phosphotyrosine is detected with an anti-phosphotyrosine-HRP antibody conjugate and subsequent color development with K-Blue reagents. All assays are optimized to use the least amount of enzyme necessary for a reproducible signal-to-noise ratio[1].

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

---

## Product Data Sheet

---

### Cell experiment:

K-562 cells are grown in RPMI 1640 supplemented with 10% fetal calf serum (FCS), and 50 g/mL Gentamycin. Meg-01 cells are cultured in Vitacell modified RPMI 1640 (ATCC), supplemented with 10% FCS and 50 µg/mL Gentamycin. The human GM-CSF-dependent myeloid leukemia cell line TF-1 is grown in RPMI 1640 supplemented with 10% FCS, 50 µg/mL Gentamycin, and 1 ng/mL of recombinant human GM-CSF. DAGM murine myeloid leukemia cells are cultured in RPMI 1640 supplemented with 10% FCS, 50 µg/mL Gentamycin, and 0.5 ng/mL recombinant IL-3. Concentrated stock solutions of PP2 (5 mM) and A-419259 (2 mM) are prepared in DMSO and stored at -20°C. Cellular proliferation is measured using the Live/Dead growth assay. This assay employs calcein-AM, a fluorogenic esterase substrate that is taken up by viable cells and hydrolyzed intracellularly, releasing a green fluorescent product. Briefly, 10<sup>4</sup> cells are plated per well in 96-well plates for each day of a 4-day time course. Various concentrations of PP2, A-419259 or vehicle control are added to the wells (five wells per concentration per day) and the plates are incubated at 37°C. At each time point, one plate is centrifuged at 1500 g for 10 min to pellet the cells. Cells are washed with phosphate buffered saline (PBS), and calcein-AM is added to each well to a final concentration of 1 µM. Plates are incubated in the dark at room temperature for 1 h. The plates are then read at 485/530 nm (excitation/emission) using a SpectraMax Gemini XS fluorescent plate reader and data are analysed with SoftMax Pro software[1].

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

---

## Product Data Sheet

---

### References:

[1]. Wilson MB, et al. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis.

Oncogene. 2002 Nov 21;21(53):8075-88.

[2]. Pene-Dumitrescu T, et al. An inhibitor-resistant mutant of Hck protects CML cells against the antiproliferative and apoptotic effects of the broad-spectrum Src family kinase inhibitor A-419259. Oncogene (2008) 27, 7055-7069

### Background

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA

---

## Product Data Sheet

---

A-419259 is an inhibitor of Src family kinases, including Src, LCK, Lyn, and Hck ( $IC_{50}$ s = 9, <3, <3, and 11.26 nM, respectively).<sup>1,2</sup> It is selective for these kinases over c-Abl ( $IC_{50}$  = 3,000 nM) and PKC ( $IC_{50}$  = >33  $\mu$ M).<sup>1</sup> A-419259 inhibits growth of Philadelphia chromosome-positive (Ph<sup>+</sup>) K-562 and Meg-01 myeloid leukemia cells ( $IC_{50}$ s = 0.1-0.3 and 0.1  $\mu$ M, respectively), but not Ph<sup>-</sup> TF-1 and HEL cells. It induces apoptosis in K-562 cells in a concentration-dependent manner. A-419259 (300 nM) inhibits differentiation of murine embryonic stem cells while maintaining pluripotency.<sup>3</sup> It reduces the total number of acute myeloid leukemia (AML) cells, as well as AML stem cells, in the bone marrow and spleen in mouse patient-derived xenograft (PDX) models of AML when administered at a dose of 30 mg/kg twice daily.<sup>4</sup>

1. Wilson, M.B., Schreiner, S.J., Choi, H.J., et al. Selective pyrrolo-pyrimidine inhibitors reveal a necessary role for Src family kinases in Bcr-Abl signal transduction and oncogenesis *Oncogene* 21(53):8075-8088(2002)  
2. Pene-Dumitrescu, T., Peterson, L.F., Donato, N.J., et al. An inhibitor-resistant mutant of Hck protects CML cells against the antiproliferative and apoptotic effects of the broad-spectrum Src family kinase inhibitor A-419259 *Oncogene* 27(56):7055-7069(2008)  
3. Meyn, M.A., III, Schreiner, S.J., Dumitrescu, T.P., et al. SRC family kinase activity is required for murine embryonic stem cell growth and differentiation *Mol. Pharmacol.* 68(5):1320-1330(2005)  
4. Saito, Y., Yuki, H., Kuratani, M., et al. A pyrrolo-pyrimidine derivative targets human primary AML stem cells in vivo *Sci. Transl. Med.* 5(181):181ra52(2013)

**Caution: Product has not been fully validated for medical applications. For research use only.**

Tel: (909) 407-4943 Fax: (626) 353-8530 E-mail: tech@glpbio.com

Address: 10292 Central Ave. #205, Montclair, CA, USA