

Protein Kinase Inhibitor/Activator Preparation Instructions.

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The range of compounds used to modify protein kinase and phosphatase activity in cell-based assays is very wide. This database is intended to help the user select an appropriate compound and give initial directions for use. As with any chemical inhibitor or activator, care must be taken that the concentration is appropriate and specific for your use. And for most cell based assays, it is important to determine that the effects observed are due to the action of the compound. One means to do this is by using more than one inhibitor each with different mechanisms of action. *Cohen P (2003) Celltransmissions, Sigma Tech bul. 19:11.* Literature range referenced here are the most common concentrations for various cell lines. Note that while some compounds may be handled differently, when possible a stock solution has been suggested that will store at -20°C.

Key Words: Protein kinase, inhibitors, activators

Key internet site: The protein kinase resource (<http://pkr.sdsc.edu/html/index.shtml>)
Calbiochem Technical Resources (<http://www.Calbiochem.com>)

Compound	Description	MW	Literature Range	Reference	Suggested Stock Concentration	Suggested Directions for Use
A23187 / calcimycin	A mobile ion-carrier that forms stable complexes with divalent cations which can permeabilize the plasma membrane and induce the leakage of Ca ²⁺ . Useful for increasing concentration of intracellular Ca ²⁺ . Can increase Ox Phos by uncoupling and inhibits mitochondrial ATPase. Also inhibits HSP70 gene expression.	523.6	0.5 - 10 μ M	Bennardini F et al. (1995) Biochem. Biophys. Res. Commun. 208: 742-747	25 mM or 13.1 mg/ml 76.39 μ l DMSO per 1.0 mg.	Prepare by rapid mixing (5 μ l in 2.5 ml of media in 0.1% FFA BSA = 50 μ M). Will aggregate over time in aqueous solution. Quickly add 10 μ l of the 50 μ M solution/ml of cells.
AG 9	Phenolic (tyrosine analogue) compound that poorly inhibits protein tyrosine kinases. Serves as a control for other AG compounds (particularly AG490) IC ₅₀ >1250 μ M for EGFR kinase.	184.2	50 μ M	Gazit, A., et al. (1989) J. Med. Chem. 32: 2344	54.3 mM or 10 mg/ml in DMSO, acetic acid or ethanol.	Dilute stock 1:5 with solute. Use 4.6 μ l/ml of cells.
AG 490	Inhibitor of EGFR auto-phosphorylation (IC ₅₀ = 100 nM) also a Jak-2 and STAT inhibitor.	294.3	30-60 μ M	Meydan N. et al. (1996) Nature 379: 645	20 mM or 5.88 mg/ml 20 μ l / tube. Stock Prep - Add 0.849 ml DMSO to 5 mg vial.	Assay Prep: Dilute stock 1:4 w/ DMSO (5 mM). Use 10 μ l/ml of cells. 15 - 30 min preincubation
AG 1296	Tyrphostin derivative (tyrosine structural analogs) that acts at the active site of the kinase. PDGFR inhibitor (IC ₅₀ = 0.5 μ M) EGFR IC ₅₀ > 100 μ M. Used as a control to EGFR activation / inhibition	266.3	0.3 -3 μ M	Kovalenko, M., et al. (1997) Biochemistry 36: 6260	20 mM or 5.35 mg/ml 20 μ l per tube. Stock Prep - Add 0.935 ml DMSO to 5 mg vial.	Assay Prep: Dilute stock (20 mM) 1:20 in DMSO and 1:10 w/ serum free media containing 0.1 % fatty acid free BSA (200 μ M). Use 10 μ l/1.0 ml of cells. 15 - 30 min preincubation
AG 1478	Highly specific and selective EGFR kinase inhibitor (IC ₅₀ = 3 nM). Blocks autophosphorylation. Competitive ATP inhibitor	315.8	0.1 - 1 μ M	Levitzki, A et al. (1995) Science 267: 1782	20 mM or 6.33 mg/ml 20 μ l / tube. Stock Prep - Add 0.79 ml DMSO to 5 mg vial.	Assay Prep: Dilute (20 mM) 1:100 in DMSO and then 1:10 w / serum free media containing 0.1 % fatty acid free BSA. (200 μ M). Use 5 μ l/1.0 ml of cells. 15 - 30 min preincubation
Anisomycin	Activates p54 and selective MAP kinases. Involved in the	265.3	20 - 50 ng /ml, 200 -	KyriakisJM., et al (1994)	0.2 mM - Dilute 2 mM 1:10 in DMSO.	- add 1 μ l of 0.2 mM/ml of cells

	activation of stress MAP kinases (P38 and JNK). An inhibitor of protein synthesis at the translation step - ribosome and elongation.		500—ng, ml and 10 - 50 $\mu\text{g}/\text{ml}$ for 15 - 120 min and 2 - 7 hrs	Nature 369: 156	2 mM- Dilute 100 mM 1:50 in DMSO. 100 mM (26.5 mg/ml).	- add 1 μl of 2 mM/ml of cells - add 1 μl of 100 mM/ml of cells
Bisindolyl-maleimide I (BIM)	Selective PKC inhibitor ($K_I = 10$ nM) Is a derivative of the less specific compound, staurosporine. Acts as a competitive inhibitor for the ATP-binding site of PKC. Highly selective for α , β_I , β_{II} , γ , δ and ϵ isozymes. May inhibit PKA at 1 μM or higher.	412.5	0.5 - 5 μM	Hers, I., et al. 1999. FEBS Lett. 460, 433	2.42 mM or 1 mg/ml. 5 μl / tube. Stock Prep - Add 100 μl per 100 μg of inhibitor.	Assay Prep: Do not dilute stock. Use 1 μl /1.0 ml cells.
Genistein	This is a broad range tyrosine kinase inhibitor EGFR and p60 ^{src} IC_{50} 20 - 25 μM PKC and PKA IC_{50} >350 μM	270.2	150-200 μM	Akiyama T et al (1987) JBC 262: 5592 - 5595	100 mM or 27 mg/ml 20 μl /tube. Stock Prep - Add 0.74 ml DMSO to 20 mg vial.	Assay Prep: Directly add 2.0 μl /ml of cells. Add slowly and swirl while adding. Some will come out of solution. 15 - 30 min preincubation
Gö 6976	Member of the Indolocarbazole inhibitor family. Strong inhibitor of PKC ($\text{IC}_{50} = 2-7$ nM), specifically Ca^{2+} dependent forms (particularly PKC α). Does not inhibit other isoforms at μM concentrations.	378.4	0.1 - 5 μM	Martiny-Baron GM et al (1993) JBC 268: 9194	5 mM or 1.89 mg/ml. 10 μl / tube. Stock Prep - Add 52.85 μl per 100 μg of inhibitor.	Assay Prep: Dilute stock 1:50 with DMSO (0.1 mM). Use 5 μl /1.0 ml cells.
H ₂ O ₂	General inhibitor of protein tyrosine phosphatases. Induces rapid tyrosine phosphorylation of numerous cellular proteins	34	0.5 - 5 mM	Bae YS (1997) JBC 272: 217 - 221	Store as undiluted 30 - 37% solution. Density = 1.19 g/cm ³	Assuming a 30% starting solution (0.35 M), dilute 1:1000 and use 2.9 μl /ml of cells. Dilute immediately before use.
LY 294002	A more specific PI3 Kinase inhibitor than wortmannin. Reversibly competes with ATP. ($\text{IC}_{50} = 1.4$ μM). Poorly inhibits DAG kinase, PKC, MAPK and other kinases at 50 μM .	307.4	2 - 50 μM	Vlahos, C.J. et al (1994) JBC 269: 5241-5248	65.1 mM or 20 mg/ml DMSO or ethanol. Stable for only about one month once in solution.	Dilute stock 1:10 with DMSO or EtOH. Use 3.1 μl of diluted stock /ml of cells (20 μM final concentration).
Ortho Vanadate	Non-specific protein tyrosine phosphatase inhibitor. Procedure is described to depolymerize the compound to achieve optimum	183.9	0.05 - 0.5 mM	Gordon, J., (1991) Methods Enzymol. 201: 477-482	1. Prepare a 100 mM solution of sodium orthovanadate. 2. Adjust the pH to	Use 1 μl /ml of cells for a final concentration of 0.1 mM.

	inhibition of protein tyrosine phosphatases. Can be converted to pervanadate by hydrogen peroxide.			& Upstate Signaling Protocol	10.0 using either 1 N NaOH or 1 N HCl. At pH 10.0 the solution will be yellow. 3. Boil the solution until it turns clear. Then cool. 4. Readjust the pH to 10.0 and repeat step 3 until the solution remains clear and the pH stabilizes at 10.0. 5. Store aliquots at -20°C.	
PD 98059	A flavone derived compound. Selective inhibitor of MAPKK (MEK). IC ₅₀ = 2 - 10 μM. It acts by binding to the inactivated form of MEK, thereby preventing its phosphorylation by c-Raf or MEK kinase. Specific for MEK vs other tyrosine kinases. Noncompetitive inhibitor with respect to both MEK substrates, ATP and ERK.	267.3	2 - 150 μM	Dudley DT et al (1995) Proc. Natl. Acad. Sci USA. 92, 7686-7689	20 mM or 5.35 mg/ml in DMSO.	Assay Prep: Use 5 μl/ ml of cells.
PP2	Src family tyrosine kinase inhibitor.. Binds to a threonine residue shared among Src kinases but absent in most other nonreceptor tyrosine kinases. Selectively inhibits lck and fyn (IC ₅₀ = 4 - 5 nM). EGFR IC ₅₀ = 480 nM	301.8	1 - 20 μM	Salazar EP et al (1999) JBC 274: 28371	10 mM or 3 mg/ml 20 μl / tube. Stock Prep - Add 0.333 ml DMSO to 1 mg vial.	Assay Prep: Dilute stock (10 mM) 1:5 w/ DMSO. Use 5 μl/1.0 ml of cells. - often times this is incubated for an hour prior to agonist addition
PMA (Phorbol-12-myristate-13- acetate)	Extremely potent skin tumor promotor. Mimics action of Diacyl glycerol. Activates PKC at nM concentration. Most commonly used phorbol ester.	616.8	1-100 nM	Nishizuka Y (1986) Science 233: 305-312	1.62 mM in DMSO. Or 1 mg/ml. 20 μl / tube	Dilute 9.5μl stock PMA with 514 μl media = 30 μM. Use 3.33 μl /ml of cells.
Ro-31-8220	Specific PKC inhibitor (IC ₅₀ = 10 nM) vs PKA (IC ₅₀ = 900 nM) and	553.7	0.5 - 10 μM	Beltman J et al (1996) JBC	2.5 mM or 1.8 mg/ml. 5 μl / tube.	Assay Prep: Do not dilute stock. Use 2 μl/1.0 ml cells.

	Ca ²⁺ /CamKinase (IC ₅₀ = 17 μM) Inhibits active membrane bound PKC 12.5 times better than cytosolic PKC. Staurosporine analogue.			271: 27018	Stock Prep - Add 72.24 μl per 100 μg of inhibitor.	
SB 203580	A pyridinylimidazole compound, p38 is specifically inactivated through binding in the ATP pocket. Inhibits an inhibitor of JNK and p38/HOG kinase with in vitro IC ₅₀ values of 0.3-0.6 μM. No or little effect on other MAP kinases	377.4	0.1 - 20 μM	Cuenda, A et al (1995) FEBS Lett. 364: 229-233	30 mg/ml or 79.5 mM in DMSO.	Assay Prep: Dilute stock 1:10 with DMSO, then use 1.25 μl/ml of cells.
SB-216763	GSK-3 β (and α) inhibitor (IC ₅₀ = 34.). No significant inhibition of PKB, PDK1 or CDK-2. Mimics insulin and decreases neuronal death by PI-3K.	371.2	0.1 - 5 μM	Coghlan MP et al (2000) Chem. Biol. 7:793-803	53.9 mM or 20 mg/ml in DMSO	Dilute stock 1:50 and use 2 μl / ml of media.
Staurosporine	A broad-spectrum kinase inhibitor. CaM Kinase (IC ₅₀ = 20 nM), PKA (IC ₅₀ = 7 nM) PKC (IC ₅₀ = 0.7 nM), PKG (IC ₅₀ = 8.5 nM). Also inhibits tryosine kinases. High dose and long term treatment results in cell death in several cell lines.	466.5	2 - 10 μM	Nishimura H et al (1994) Biochem. J. 302: 271	5 mM or 2.33 mg/ml. 8 μl / tube. Stock Prep - Add 42.87 μl per 100 μg of inhibitor. Store at 4°C for 6 months	Assay Prep: Dilute stock Do not dilute. Use 1.0 μl/ml of cells.
U0125	Similar but less potent version of U0126.	350.5	5 - 100 μM	Favata et al (1998)JBC 273: 18623 - 18632.	10 mM or 3.805 mg/ml; 0.263 ml DMSO per mg.	Assay Prep: Add 2.5 μl / ml of cells. Stable for 1-2 weeks at -20°C
U0126	First found as an AP-1 inhibitor. Noncompetitive inhibitor of MEK with respect to ATP and ERK. IC ₅₀ = 58 - 75 nM. Little inhibition of PKC, Abl, Raf, MEKK, ERK, JNK, MKK-3, MKK-4/SEK, MKK-6, Cdk2, or Cdk4. Little if any affect on PKC.	380.5	1 - 50 μM	Favata et al (1998)JBC 273: 18623 - 18632.	1) 10 mM or 3.805 mg/ml; 0.263 ml DMSO per mg. 2) Dissolve 1 mg in 0.263 ml chloroform, divide into 50 μl aliquot and dry under N ₂ . Just prior to use reconstitute to 50 μl with DMSO.	Assay Prep: Add 2 μl/ml of cells. Unstable in solution. Use within 2 hours of preparation.

Y-27632	A highly potent cell-permeable pyridine derivative, which is a selective inhibitor of Rho-associated protein kinases (IC_{50} = 700 nM, K_i = 140 nM for ROCK). Exhibits 50 to 100 fold lower affinity for $PKC_{\alpha,\epsilon}$ than p160ROCK. Does not significantly affect the activity of PAK or Cdc42. its activity against myosin light chain kinase is 2000-fold lower than ROCK	338.3	5 - 20 μ M	Uehata, M (1997) Nature 389: 990-994	20 mM or 6.77 mg/ml 5 μ l/tube. Stock Prep - Add 0.148 ml DMSO to 1 mg vial.	Assay Prep: Do not dilute stock. Use 1 μ l/1.0 ml cells.
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