

TECHNICAL APPENDIX

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Western Blot Analysis with Anti-ACTIVE® MAPK, JNK and p38 pAbs

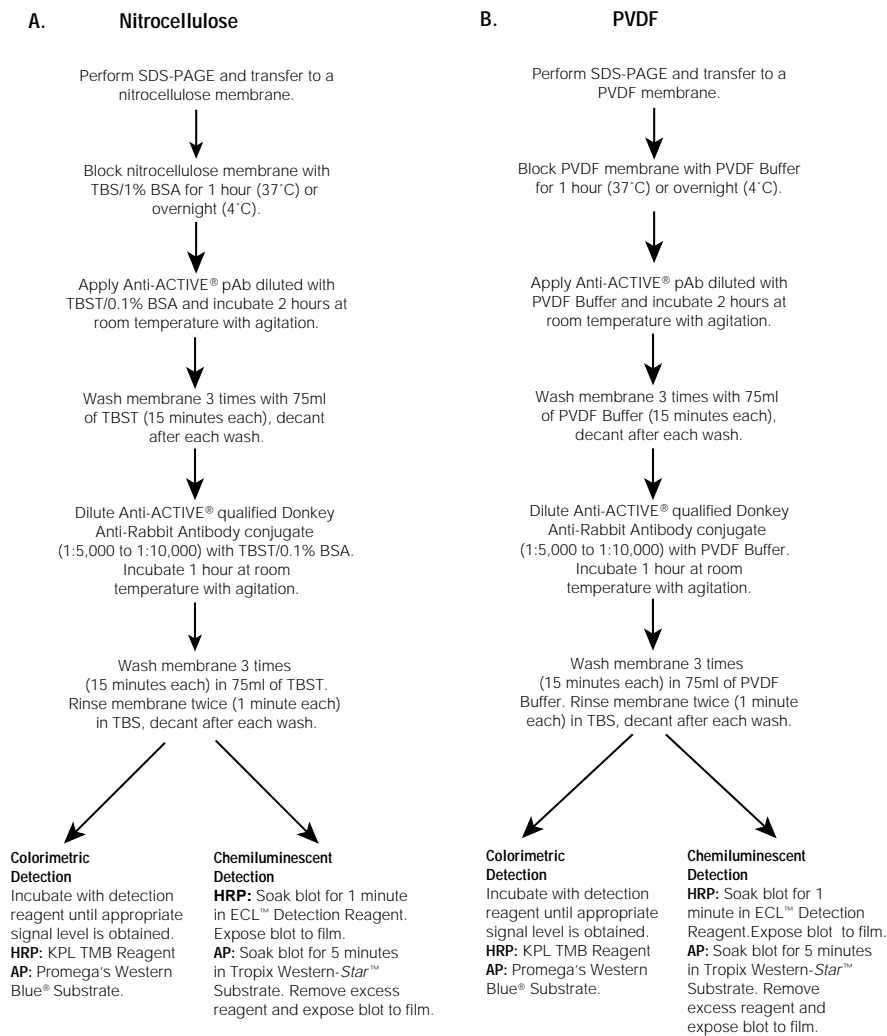


Figure 10.1. Schematic diagram illustrating the use of nitrocellulose and PVDF membranes in Western blot analysis with Anti-ACTIVE® pAbs. Protocols for use with nitrocellulose (Panel A) and PVDF (Panel B) membranes. Recommended dilutions of the Anti-ACTIVE® pAbs are 1:5,000 for Anti-ACTIVE® MAPK pAb, 1:2,000 for Anti-ACTIVE® p38 pAb, 1:5,000 for Anti-ACTIVE® JNK pAb and 1:5,000 to 1:10,000 for the Anti-ACTIVE® Qualified Donkey Anti-Rabbit IgG (H+L) secondary antibodies (both HRP- and AP-conjugated). KPL is an abbreviation for Kirkegaard and Perry Laboratories. See Technical Bulletin #TB262 for more information about this protocol.

Note: It may be necessary to empirically determine the optimal dilutions of primary and secondary antibodies for your system. Use of secondary antibodies other than those available from Promega may require additional optimization.

Composition of Buffers

TBS buffer

20mM	Tris-HCl (pH 7.5)
150mM	NaCl

PVDF buffer

TBS buffer with 0.2% I-Block™ (Tropix) and 0.1% Tween® 20

TBST buffer

TBS buffer with 0.05% Tween® 20

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Immunocytochemistry with Anti-ACTIVE® MAPK, JNK and p38 Abs

The following method is for preparing and immunostaining PC12 cells stimulated by either nerve growth factor (NGF) to activate MAP kinases or sorbitol to activate JNK and p38 kinases. For more information about this protocol, see Technical Bulletin #TB262.

Materials to Be Supplied by the User

- Lab-Tek® 4-chambered slides (Fisher Cat.# 12-565-21)
- rat tail collagen (Collaborative BioScience Products)
- RPMI 1640 with 25mM HEPES, 300mg/L L-glutamine, 10% horse serum, 5% fetal bovine serum and 0.5mM EGTA.
- NGF (Promega Cat.# G5141) or sorbitol
- PBS
- 10% paraformaldehyde
- methanol (-20°C)
- blocking buffer
- Donkey Anti-Rabbit Cy™3 conjugate (Jackson ImmunoResearch Cat.# 741-165-152)

Preparation and Activation of PC12 Cells

1. Coat Lab-Tek® 4-chambered slides with rat tail collagen (6µg/cm² in sterile PBS) for one hour.
2. Grow PC12 cells in chambers at 37°C in 5% CO₂ in medium containing RPMI 1640 with 25mM HEPES, 300mg/L L-glutamine, 10% horse serum, 5% fetal bovine serum and 0.5mM EGTA. The medium should be changed every other day until the cells reach 80% confluence.
3. Activate the cells in 2 chambers as described below. Use the cells in the remaining 2 chambers as untreated controls.

NGF Treatment to Activate ERK 1/2

The day before immunocytochemistry (ICC), add fresh medium with serum. The next day, add 200ng/ml NGF in RPMI. Incubate for 5 minutes at 37°C.

Sorbitol Treatment to Activate JNK and p38 Kinase

The day before ICC, add fresh medium without serum. The next day add sorbitol to a final concentration of 1M. Incubate for 30 minutes at 37°C.

4. See Figure 10.2 for immunostaining procedure.

Composition of Buffers

TBS buffer

20mM Tris-HCl (pH 7.5)
150mM NaCl

10% paraformaldehyde

5g paraformaldehyde
50ml PBS

blocking buffer

1% BSA and 5% donkey serum in PBS

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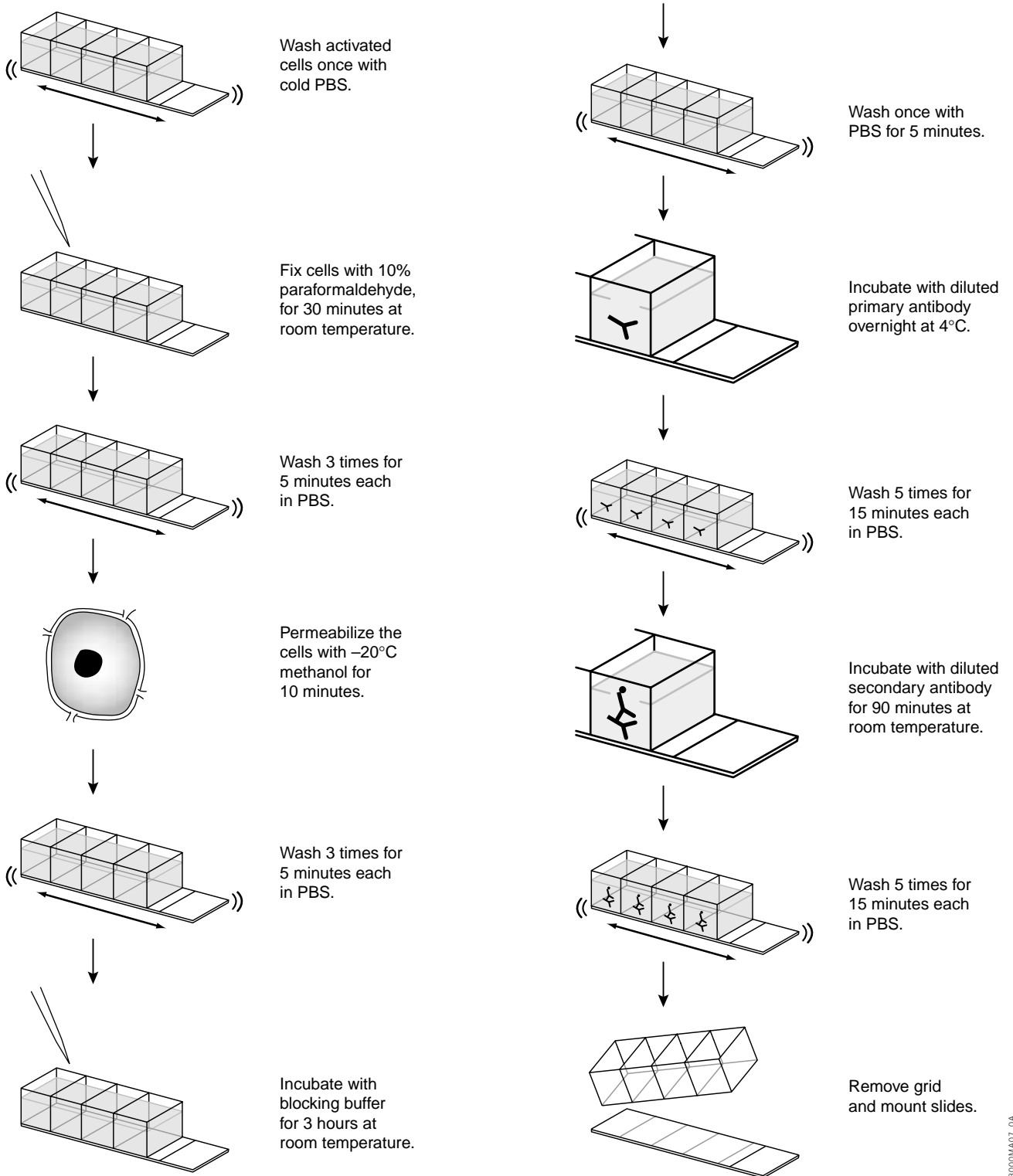
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Figure 10.2. Immunostaining of activated PC12 cells.



(continued in next column)

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References

1. Meier, R. *et al.* (1996) Cellular stresses and cytokines activate multiple mitogen-activated-protein kinase kinase homologues in PC12 and KB cells. *Eur. J. Biochem.* **236**, 796.
2. Xia, Z. *et al.* (1995) Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270**, 1326.
3. Boulton, T.G. *et al.* (1991) Purification and properties of extracellular signal-regulated kinase 1, an insulin-stimulated microtubule-associated protein 2 kinase. *Biochem.* **30**, 278.

Generation of Control PC12 Cell Extracts

PC12 (pheochromocytoma) cell extracts can be prepared using the following procedure, for use as positive and negative controls for Western blot analysis with the Anti-ACTIVE® pAbs. PC12 cells are grown to an appropriate density, then treated with either NGF or sorbitol, or are left untreated. NGF will potently activate ERK isoforms but not JNK or p38 kinases. Sorbitol, on the other hand, will induce the activation of ERK, JNK and p38 by osmotic stress. The extracts are harvested and stored at -70°C for future use.

Materials to be Supplied by the User

- PC12 cells (ATCC# CRL1721)
- RPMI 1640 medium, supplemented (Gibco BRL Cat.# 22400-089)
- 25, 75 and 150cm² tissue culture flasks (Corning® Cat.# 25100-25, 25110-75 and 25126-150)
- rat tail collagen (Sigma Cat.# C7661)
- 37°C incubator with 5% CO₂
- cell scraper (Costar® Cat.# 3010)
- sorbitol (Sigma Cat.# S3889)
- NGF (nerve growth factor; Promega Cat.# G5141)
- Dulbecco's PBS without Ca²⁺ and Mg²⁺ (Gibco BRL Cat.# 14200-075)
- Dounce type A glass homogenizer (Fisher Cat.# K885300-0015)
- harvest buffer

1. Precoat 25cm² tissue culture flasks with rat tail collagen, 6µg/cm² in 3ml of RPMI 1640 medium without serum, at 37°C for at least one hour. Remove the excess liquid and store the flasks at 37°C, 5% CO₂ until the next day.
2. Grow PC12 cells in RPMI 1640 medium, supplemented. Change the medium every day until the cells reach 80% confluence, as determined by inspection with an inverted light microscope. Using a cell scraper, scrape the cells to dislodge from the flask. Resuspend in 30ml of fresh RPMI 1640 medium, supplemented, in a 75cm² flask.
3. When the cells in the 75cm² flask reach 80% confluence, scrape the cells to dislodge from the flask, resuspend in 240ml of fresh RPMI 1640 medium, supplemented, and dispense into four 150cm² flasks (total volume of 60ml/150cm² flask). Allow the PC12 cells to reach 80% confluence in the 150cm² flasks.
4. On the day before stimulation and harvest, add fresh medium **with** serum to the flasks for the negative control or NGF treatment, and fresh medium **without** serum to the flasks for sorbitol treatment. Proceed to either Step 5 for sorbitol stimulation or Step 6 for NGF stimulation.
5. **Sorbitol-treated cells:** On the day of stimulation, add 2.5M aqueous sorbitol solution to the flasks in order to achieve a final concentration of 0.5M sorbitol (1). Stimulate for 5 minutes, incubating at 37°C. Proceed to Step 7 for harvesting the cells.
6. **NGF-treated cells:** On the day of stimulation, add NGF at 50ng/ml final concentration in the flask and stimulate cells for 5 minutes at 37°C (2). Proceed to Step 7 for harvesting the cells.
7. To harvest the cells, remove the media and rinse the cell monolayer once with 10ml of ice-cold Dulbecco's PBS without Ca²⁺ and Mg²⁺. Add 2ml of ice-cold harvest buffer per 150cm² flask and scrape the cells. Transfer the cell suspension to a Dounce type A glass homogenizer on ice.
8. Break the cells open, on ice, with 125 strokes of the homogenizer, then centrifuge the lysed cell suspension in a microcentrifuge for 2 minutes at 10,000 × g, 4°C. Centrifuge the resulting supernatant solution in an ultracentrifuge for 1 hour at 97,000 × g, 4°C.
9. Aliquot the final supernatant to 50µl per tube. Store at -70°C.
10. Determine the protein concentration of the extracts.

Composition of Buffers

RPMI 1640 medium, supplemented

25mM	HEPES
2mM	L-glutamine
10%	horse serum
5%	fetal bovine serum
50 units/ml	penicillin
50µg/ml	streptomycin
0.5 mM	EGTA

harvest buffer (3)

20mM	Tris-HCl (pH 7.3)
20mM	p-nitrophenyl phosphate
1mM	EGTA
50mM	NaF
50µM	sodium o-vanadate
5mM	benzamidine

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Frequently Asked Questions for Anti-ACTIVE® MAPK, JNK and p38 pAbs and MAPK Signaling Pathways

Exactly what are "Anti-ACTIVE®" Antibodies?

Promega's Anti-ACTIVE® antibodies are designed to specifically recognize the active forms of particular enzymes. Typically, enzymes are activated through post-translational modification—usually phosphorylation. In the MAPK signaling pathway, the kinases MAPK, JNK and p38 each contain a Thr-X-Tyr consensus sequence (see Table 10.1) in the "phosphorylation loop" within the catalytic domain. The kinases are active only when both the threonine and tyrosine residues of this sequence are phosphorylated. Promega's family of MAPK Anti-ACTIVE® Antibodies are raised against the phosphorylated peptide core sequences and are specific to the active, dually phosphorylated forms of MAPK, JNK and p38. Minimal cross-reactivity occurs with monophosphorylated and nonphosphorylated forms of the proteins or between members of this superfamily.

How are the Anti-ACTIVE® Antibodies purified?

The Anti-ACTIVE® Antibodies are purified through a two-step process. Initially, the rabbit serum is immunodepleted by passage over a column containing the unphosphorylated peptide. This is followed by affinity chromatography over a column containing the phosphorylated immunogen peptide that corresponds to the active form of the kinase. This process results in the production of a highly specific polyclonal antibody preparation that is highly reactive to only the dually phosphorylated enzyme.

Why use Anti-ACTIVE® Antibodies?

Promega's Anti-ACTIVE® Antibodies are tested to work in Western analysis with crude cell extracts and thus provide a simple, non-radioactive method to detect active kinase. In addition, the MAPK family of Anti-ACTIVE® Antibodies are tested for use in immunostaining applications—every lot of antibody is tested for activity on PC12 cells (rat pheochromocytoma cells), which express activated ERK (MAPK), JNK or p38 kinases. Therefore, Anti-ACTIVE® Antibody-based assays can replace other methods of detecting active kinase such as electrophoretic mobility shift assays, in-gel kinase assays, immunoprecipitation-based kinase assays and Western blotting with anti-phosphotyrosine antibodies. The MAPK family of Anti-ACTIVE® Antibodies are supplied with complete protocols for Western analysis and immunocytochemistry.

Which secondary antibodies should be used with Anti-ACTIVE® Antibodies?

Promega's Anti-ACTIVE® Antibodies are polyclonal antibodies isolated from rabbit serum. Therefore, anti-rabbit IgG secondary antibodies should be used for detection. Appropriate controls consisting of secondary antibody only (e.g., no primary antibody) are recommended to determine any contribution the secondary antibody may make to background signal. Promega offers horseradish peroxidase- and alkaline phosphatase-conjugated Donkey Anti-Rabbit IgG secondary antibodies (Cat.# V7951 and V7971) designed for use with the Anti-ACTIVE® Antibodies. These Anti-ACTIVE®-qualified antibody conjugates have been certified to perform optimally with the primary Anti-ACTIVE® Antibodies and to give highly specific signals and low background in Western blot analysis of crude cell extracts using chemiluminescent or colorimetric detection. They are also optimized for minimum cross-reactivity with immunoglobulin G from other species.

What is the MEK Inhibitor U0126 and how does it compare to PD 98059?

MAP kinase kinase 1 and 2 (MEK 1/2) are the enzymes responsible for activating ERK 1/2. MEK Inhibitor U0126 (Cat.# V1121) is a novel, selective and potent inhibitor of MEK 1/2. U0126 is an organic compound (1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene; MW= 426.5) that binds to MEK in a noncompetitive manner and prevents the enzyme from phosphorylating ERK by inhibiting the catalytic activity of the active enzyme. The MEK inhibitor PD 98059 (Cat.# V1191), on the other hand, prevents MEK1 activation by Raf (PD 98059 is less active against MEK2). PD 98059 binds to inactive MEK1 and prevents Raf from phosphorylating it; however, PD 98059 has little effect on active MEK1. Thus, U0126 will inhibit downstream activation of ERK 1/2 regardless of the activation state of MEK 1/2. Additionally, U0126 is a more potent inhibitor than PD 98059 both in vitro and in vivo. U0126 inhibits constitutively active MEK1 with an IC₅₀ of 0.07 μM, while PD 98059 inhibits at an IC₅₀ of 10 μM (1). IC₅₀ results may vary depending on the kinase and cell type used.

Does Promega offer any other MAPK signaling inhibitors?

In addition to U0126 and PD 98059, Promega offers SB 203580, a specific, cell-permeable inhibitor of the p38 kinase isoforms, p38α, p38β and p38β-2. SB 203580 acts as a competitive inhibitor of ATP binding. Reported IC₅₀ values for p38 activity range from 21 nM to 1 μM (2–5). SB 203580 has no significant effect on the activities of ERKs, JNKs, p38γ or p38δ.

References

1. Favata, M. *et al.* (1998) *J. Biol. Chem.* **273**, 18623.
2. Henry, J.R. *et al.* (1998) *Bioorg. Med. Chem. Lett.* **8**, 3335.
3. Young, P.R. *et al.* (1997) *J. Biol. Chem.* **272**, 12116.
4. Gallagher, T.F. *et al.* (1997) *Bioorg. Med. Chem.* **5**, 49.
5. Cuenda, A. *et al.* (1995) *FEBS Lett.* **364**, 229.

Table 10.1. Core Amino Acid Sequences Recognized by Promega's family of MAPK Anti-ACTIVE® Antibodies.

Anti-ACTIVE® Antibody	Catalytic Core
MAPK	pThr ¹⁸³ -Glu ¹⁸⁴ -pTyr ¹⁸⁵
JNK	pThr ¹⁸³ -Pro ¹⁸⁴ -pTyr ¹⁸⁵
p38	pThr ¹⁸⁰ -Gly ¹⁸¹ -pTyr ¹⁸²

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Protein Kinase Substrates and Inhibitors

Table 10.2. Peptide Substrates for Selected Protein Kinases.

Kinase	Molecular Weight	Substrate Sequence ^a	K _m
cdc2 Kinase	1137Da	Pro-Lys- Thr -Pro-Lys-Lys-Ala-Lys-Lys-Leu	5μM (1)
Casein Kinase I	1235Da	Asp-Asp-Asp-Glu-Glu- Ser -Ile-Thr-Arg-Arg	0.5–1.0mM (2)
Casein Kinase II	1362Da	Arg-Arg-Arg-Glu-Glu-Glu- Thr -Glu-Glu-Glu	0.5mM (3)
DNA-Dependent Protein Kinase	1759Da	Glu-Pro-Pro-Leu- Ser -Gln-Glu-Ala-Phe-Ala-Asp-Leu-Trp-Lys-Lys	760μM (4)
Protein Kinase A (Kemptide)	772Da	Leu-Arg-Arg-Ala- Ser -Leu-Gly	16μM (5)
Protein Kinase C (Neurogranin ₂₈₋₄₃)	1800Da	Ala-Ala-Lys-Ile-Gln-Ala- Ser -Phe-Arg-Gly-His-Met-Ala-Arg-Lys-Lys	150nM (6)
Protein Kinase G	937Da	Arg-Lys-Ile- Ser -Ala-Ser-Glu-Phe	68μM (7)

^aThe phosphorylated residue is indicated in bold.

References

1. Beaudette, K.N., Lew, J. and Wang, J.H. (1993) Substrate specificity characterization of a cdc2-like protein kinase purified from bovine brain. *J. Biol. Chem.* **268**, 20825.
2. Agostinis, P. *et al.* (1989) A synthetic peptide substrate specific for casein kinase I. *FEBS Lett.* **259**, 75.
3. Kuenzel, E.A. and Krebs, E.G. (1985) A synthetic peptide substrate specific for casein kinase II. *Proc. Natl. Acad. Sci. USA* **82**, 737.
4. Lees-Miller, S. *et al.* (1992) Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol. Cell. Biol.* **12**, 5041.
5. Kemp, B.E. *et al.* (1977) Role of multiple basic residues in determining the substrate specificity of cyclic AMP-dependent protein kinase. *J. Biol. Chem.* **252**, 4888.
6. Chen, S.-J. *et al.* (1993) Studies with synthetic peptide substrates derived from the neuronal protein neurogranin reveal structural determinants of potency and selectivity for protein kinase C. *Biochemistry* **32**, 1032.
7. Colbran, J.L. *et al.* (1992) A phenylalanine in peptide substrates provides for selectivity between cGMP- and cAMP-dependent protein kinases. *J. Biol. Chem.* **267**, 9589.

Table 10.3. Promega Protein Kinase Inhibitors.

Inhibitor	Specificity	Molecular Weight	Stock Solution	Working Concentration
U0126 (Cat.# V1121)	Inhibits active MEK 1/2	426.5Da	10mM Dissolve 1mg in 234μl DMSO. Store in aliquots at -20°C	0.1–10μM
SB 203580 (Cat.# V1161)	p38α, p38β or p38β2	377.4Da	10mM Dissolve 1mg in 265μl DMSO. Store in aliquots at -20°C.	21nM–1μM
LY 294002 (Cat.# V1201)	Phosphatidylinositol 3-kinase (PI3K)	307.4Da	50mM Dissolve 5mg in 325μl DMSO. Store in aliquots at -20°C.	1–50μM
PD 98059 (Cat.# V1191)	Prevents MEK activation	267.3Da	20mM Dissolve 5mg in 935μl of DMSO. Store in aliquots at -20°C.	0.1–100μM
PKA Inhibitor (PKI; Cat.# V5681)	PKA	2222Da	10mg/ml Provided in water.	up to 10μM
Myristoylated PKC Inhibitor (Cat.# V5691)	PKC	1754Da	10mg/ml Provided in water.	up to 100μM
Olomoucine (Cat.# V2372)	pp34 ^{cdc2} /cyclin B (cdc2 Kinase) pp33 ^{cdk2} /cyclin A pp33 ^{cdc2} /cyclin E pp33 ^{cdk5} /p25 ERK	298.15Da	10mM Dissolve 1mg in 335μl of DMSO. Store in aliquots at -20°C.	up to 100μM

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Phosphatase Inhibitors

Table 10.4. Selected Chemical Phosphatase Inhibitors^a.

Inhibitor	Specificity ^b	Molecular Weight	Stock Solution	Working Concentration
NaF	PPase-1 n.d. PTPase – PPase-2A ++++ PPase-2B ++++ PPase-2C ++++	41.99Da	1M Dissolve in water.	50mM
Vanadate (Na ₃ VO ₄)	PPase-1 n.d. PTPase ++++ PPase-2A – PPase-2B ++ PPase-2C –	183.9Da	≤50mg/ml (270mM) Dissolve in water and adjust pH to 10. Heat to boiling until translucent. Readjust pH to 10. Solution may not completely clear. Store aliquots frozen at –20°C. ^c	1mM
Okadaic Acid	PPase-1 +++ PTPase – PPase-2A ++++ PPase-2B ++ PPase-2C –	805Da	5mM Dissolve in DMSO and store at –20°C.	12nM–5μM
EDTA	PPase-1 – PTPase – PPase-2A – PPase-2B – PPase-2C ++++	372.24Da (disodium salt)	500mM Dissolve in water and adjust pH to 8.0. Store at 4°C or room temperature. Solubility increases as pH increases.	1–20mM
EGTA	PPase-1 – PTPase – PPase-2A – PPase-2B ++++ PPase-2C –	380.4Da (free acid)	500mM Dissolve in water and adjust pH to 8.0. Store at 4°C or room temperature. Solubility increases as pH increases.	1–20mM
Trifluoperazin	PPase-1 – PTPase – PPase-2A – PPase-2B ++++ PPase-2C –	480.4Da (dihydrochloride salt)	5–20mM Dissolve in water. Store frozen aliquots at –20°C. Stable for at least 3 months.	25μM

^a*Protein Phosphorylation: A Practical Approach*. Hardie, D.G., ed., IRL Press, Oxford UK, 1993, and Cohen, P. (1991) Classification of protein-serine/threonine phosphatases: identification and quantitation in cell extracts. *Meth. Enzymol.* **201**, 389.

^b++++ high potency; +++ moderately high potency; ++ moderate potency; + low potency; – very low or no effect; n.d., not determined.

^cGordon, J.A. (1991) Use of vanadate as protein-phosphotyrosine phosphatase inhibitor. *Meth. Enzymol.* **201**, 477.

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Protease Inhibitors

Table 10.5. Commonly Used Protease Inhibitors^a.

Inhibitor	Molecular Weight	Specificity	Stock Solution	Working Concentration
PMSF	174.2Da	serine proteases	100mM in isopropanol. Stable at room temperature for at least 9 months.	0.1–1mM ^b
AEBSF	239.5Da	serine proteases	100mg/ml in water. Store aliquots at –20°C. Stable for 1–2 months.	0.1–1.0mg/ml
Benzamidine	156.6Da	serine proteases	0.3M (50mg/ml) in water. Do not store, but make fresh each time.	0.5–4.0mM
Leupeptin	475.6Da	serine proteases	1mg/ml in water. Stable for 1 week at 4°C or 6 months frozen in aliquots at –20°C.	0.5µg/ml
Pepstatin	685.9Da	aspartic proteases	1mg/ml in methanol. Stable for 1 week at 4°C or 1 month frozen in aliquots at –20°C.	0.7µg/ml
Aprotinin	6512Da	serine proteases	10mg/ml in water or aqueous buffer. At pH 7–8, stable for 1 week at 4°C or 6 months frozen in aliquots at –20°C.	0.06–2.0µg/ml
E-64	357.4Da	cysteine proteases	20mg/ml in a 1:1 mixture of ethanol and water. Stable for 1 month frozen in aliquots at –20°C.	0.5–10µg/ml
Bestatin	308.4Da	amino peptidases, exo-peptidases	5mg/ml in methanol. Stable for 6 months frozen in aliquots at –20°C.	40µg/ml
EDTA, disodium salt	372.24Da	metalloproteases	0.5M in water adjusted to pH 8.0. Stable at 4°C for at least 6 months.	0.5–1.3mM

^aThe Complete Guide for Protease Inhibition from Roche Molecular Biochemicals and *Proteins LabFax*, Price, N.C., ed., βios Scientific Publishers, Academic Press, Oxford, UK, 1996.

^bPMSF has a limited half-life in aqueous solution. For additional information on PMSF stability, see James, G. (1978) Inactivation of the protease inhibitor phenyl-methylsulfonyl fluoride in buffers. *Anal. Biochem.* **86**, 574.

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