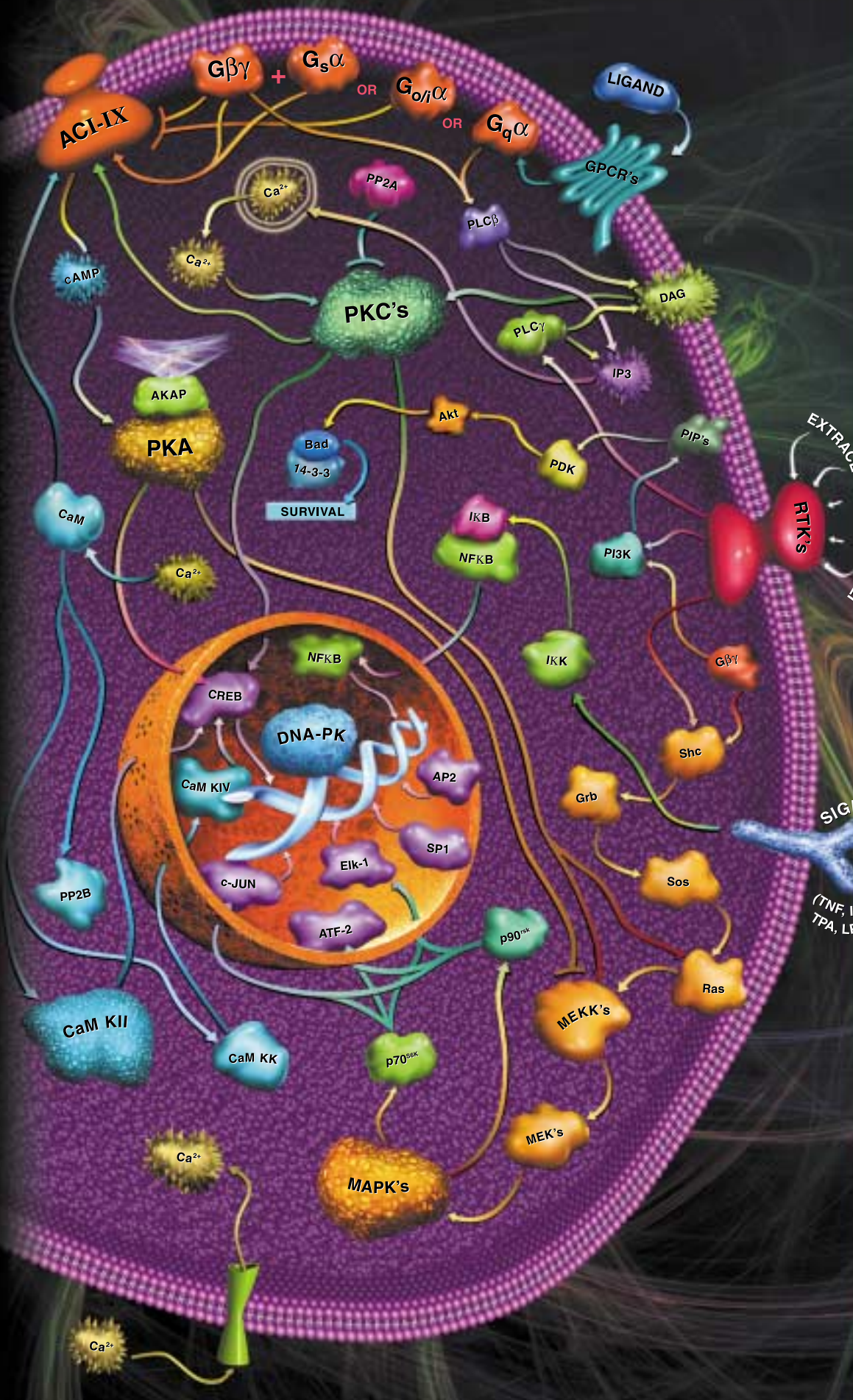


8

CHAPTER

OTHER KINASES AND REAGENTS



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Casein Kinases

Casein Kinase I

Product	Size	Catalog #
Casein Kinase I	100 units	V5631

Description: Casein Kinase I (CKI or CK-1) is an ubiquitous and highly conserved serine/threonine protein kinase found in eukaryotic cells. CKI appears to play regulatory roles in glycogen metabolism and viral viability by phosphorylation of glycogen synthase and the SV40 large T antigen, respectively. CKI exists in multiple forms in mammalian tissue and is present in the nucleus, cytosol, plasma membrane and microsomes. CKI isolated from most species is a 35–37kDa monomer. In contrast to Casein Kinase II, CKI primarily uses Mg-ATP as the phosphate donor and is not sensitive to heparin inhibition (1).

Reference

1. Tuazon, P.T. and Traugh, J.A. (1991) Casein kinase I and II—multipotential serine protein kinases: structure, function, and regulation. *Adv. Second Messenger Phosphoprotein Res.* **23**, 123.

Casein Kinase I Peptide Substrate

Product	Size	Catalog #
Casein Kinase I Peptide Substrate	1mg	V7441

Description: Casein Kinase I Peptide Substrate is a synthetic peptide substrate for casein kinase I. The sequence of the peptide is DDDEESITRR, and it is derived from the phosphorylation site of CKI in beta casein A₍₂₎ (1).

Reference

1. Agostinis, P. *et al.* (1989) A synthetic peptide substrate specific for casein kinase I. *FEBS Lett.* **259**, 75.

Casein Kinase II

Product	Size	Catalog #
Casein Kinase II	100 units	V5621

Description: Casein Kinase II (CKII or CK-2) is an ubiquitous serine/threonine protein kinase found in eukaryotic cells. CKII is known also as phosvitin kinase, glycogen synthase 5 kinase, troponin T kinase and casein kinase G. The diversity in nomenclature reflects this kinase's broad range of substrates (phosvitin, glycogen synthase, troponin T and casein, respectively) and its unique ability to utilize GTP as well as ATP as the phosphate donor. Additional names are casein kinase TS and protein kinase NII, which respectively indicate the phosphorylation targets (threonine and serine residues) and the presence of this kinase in the nucleus as well as in the cytoplasm and mitochondria. CKII is a multifunctional protein kinase that has been implicated in a variety of cellular processes and functions, including mitosis and cellular transformation.

CKII isolated from most species is composed of α and α' subunits (37–44kDa) and β subunits (24–28kDa). The holoenzyme exists as an $\alpha\alpha'\beta_2$ tetramer. The α subunit contains the catalytic domain, whereas the β subunits presumably regulate the catalytic activity of the holoenzyme.

Casein Kinase II Peptide Substrate

Product	Size	Catalog #
Casein Kinase II Peptide Substrate	1mg	V5661

Description: Casein Kinase II Peptide Substrate is a selective substrate for casein kinase II. Casein Kinase II Peptide Substrate is supplied ready for use in kinase reactions. Its sequence is RRREEETEEE (1), and its molecular weight is 1,362 daltons as verified by Fast Atomic Bombardment mass spectrometry.

Reference

1. Kuenzel, E.A. and Krebs, E.G. (1985) A synthetic peptide substrate specific for casein kinase II. *Proc. Natl. Acad. Sci. USA* **82**, 737.

Promega Product Citations

Casein Kinase I

Faundez, V.V. and Kelly, R.B. (2000) The AP-3 complex required for endosomal synaptic vesicle biogenesis is associated with a casein kinase α -like isoform. *Mol. Biol. Cell* **11**, 2591.

Yin, X., Jedrzejewski, P.T. and Jiang, J.X. (2000) Casein kinase II phosphorylates lens connexin 45.6 and is involved in its degradation. *J. Biol. Chem.* **275**, 6850.

Honda, R. and Yasuda, H. (1999) Association of p19^{ARF} with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. *EMBO J.* **18**, 22.

Casein Kinase II

Broekhuis, C.H.D. *et al.* (2000) Detailed analysis of the phosphorylation of the human La (SS-B) autoantigen. (De)phosphorylation does not affect its subcellular distribution. *Biochemistry* **39**, 3023.

Chen, M.-R. *et al.* (2000) A protein kinase activity associated with Epstein-Barr virus BGLF4 phosphorylates the viral early antigen EA-D in vitro. *J. Virol.* **74**, 3093.

Kasahara, H. and Izumo, S. (1999) Identification of the in vivo casein kinase II phosphorylation site within the homeodomain of the cardiac tissue-specifying homeobox gene product Csx/Nkx2.5. *Mol. Cell. Biol.* **19**, 526.

Casein Kinase II Peptide Substrate

Song, D.H., Sussman, D.J. and Seldin, D.C. (2000) Endogenous protein kinase CK2 participates in Wnt signaling in mammary epithelial cells. *J. Biol. Chem.* **275**, 23790.

Lee, Y., Lloyd, A.M. and Roux, S.J. (1999) Antisense expression of the CK2 α -subunit gene in *Arabidopsis*. Effects on light-regulated gene expression and plant growth. *Plant. Physiol.* **119**, 989.

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Protocol

Casein Kinase I.....**TB509**
Casein Kinase II.....**TB514**

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Promega Product Citations

cGMP-Dependent Protein Kinase

Hall, K.U. *et al.* (1999) Phosphorylation-dependent inhibition of protein phosphatase-1 by G-substrate: A Purkinje cell substrate of the cyclic GMP-dependent protein kinase. *J. Biol. Chem.* **274**, 3485.

Moon, C. *et al.* (1999) Odorants induce the phosphorylation of the cAMP response element binding protein in olfactory receptor neurons. *Proc. Natl. Acad. Sci. USA* **96**, 14605.

Schubert, R. *et al.* (1999) cAMP-dependent protein kinase is in an active state in rat small arteries possessing a myogenic tone. *Am. J. Physiol.* **277**, H1145.

Moon, C. *et al.* (1998) Calcium-sensitive particulate guanylyl cyclase as a modulator of cAMP in olfactory receptor neurons. *J. Neurosci.* **18**, 3195.

Parissenti, A.M. *et al.* (1998) Inhibitory properties of the regulatory domains of human protein kinase C α and mouse protein kinase C ϵ . *J. Biol. Chem.* **273**, 8940.

Antonsson, B. *et al.* (1997). Purification, characterization, and in vitro phosphorylation of the neuron-specific membrane-associated protein SCG 10. *Prot. Exp. Purif.* **9**, 363.

Yokoyama, C.T. *et al.* (1997) Phosphorylation of the synaptic protein interaction site on N-type calcium channels inhibits interactions with SNARE proteins. *J. Neurosci.* **17**, 6929.

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cGMP-Dependent Protein Kinase

cGMP-Dependent Protein Kinase

Product	Size	Catalog #
cGMP-Dependent Protein Kinase (α -isozyme)	6,000 units	V5171

Description: cGMP-Dependent Protein Kinase is a serine/threonine protein kinase present in smooth muscle and a variety of other tissues, including lung, heart and Purkinje cells of the cerebellum (1,2). While a definitive cellular function of cGMP-Dependent Protein Kinase has not been identified, this kinase has been implicated in the regulation of smooth muscle relaxation, platelet function, sperm metabolism, cell division and nucleic acid synthesis (1). The kinase is a 78kDa polypeptide composed of a regulatory domain and a catalytic domain and is active as a homodimer. Addition of 3 μ M cyclic GMP stimulates kinase activity approximately three fold. The enzyme is active over a broad pH range. Cellular substrates include histone proteins, the type 1 regulatory subunit of cAMP-Dependent Protein Kinase and brain G protein, a novel protein found in the mammalian cerebellum (1). cGMP-Dependent Protein Kinase has been purified to physical homogeneity from bovine lung by the method of Corbin and Doskeland (3).

References

1. Edelman, A.M., Blumenthal, D.K. and Krebs, E.G. (1987) Protein serine/threonine kinases. *Ann. Rev. Biochem.* **56**, 567.
2. Beebe, S.J. and Corbin, J.D. (1986) In: *The Enzymes*, Vol. 17, 3rd ed., Boyer, P.D. and Krebs, E.D., eds., 44.
3. Corbin, J.D. and Doskeland, S.O. (1983) Studies of two different intrachain cGMP-binding sites of cGMP-dependent protein kinase *J. Biol. Chem.* **258**, 11391.

Feature

- **Purity:** cGMP-Dependent Protein Kinase is greater than 90% pure as determined by SDS-PAGE (single band).

cGMP-Dependent Protein Kinase Peptide Substrate

Product	Size	Catalog #
cGMP-Dependent Protein Kinase Peptide Substrate	1mg	V7451

Description: cGMP-Dependent Protein Kinase Peptide Substrate is a synthetic peptide that is a specific substrate for cGMP-Dependent Protein Kinase (Cat.# V5171). Its sequence is RKISASEF, and its molecular weight is 937 daltons.

Reference

1. 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.

cGMP

Product	Size	Catalog #
cGMP, 1mM	500 μ l	V6411

Description: cGMP (Guanosine-3',5'-cyclic monophosphate) is an activator of cGMP-Dependent Protein Kinase (Cat.# V5161).

SAM²® Biotin Capture Membranes and Plates

Product	Size	Catalog #
SAM ² ® Biotin Capture Membrane	96 sample	V2861
	7.6 x 10.9cm	V7861
SAM ² ® 96 Biotin Capture Plate	96 well plate	V7541
	5 x 96 well plates	V7542

Description: The SAM²® Biotin Capture Membrane^(a) binds biotinylated molecules based on their affinity for streptavidin. The proprietary process by which the SAM²® Membrane is produced results in a high density of streptavidin on the filter, providing rapid, quantitative substrate binding in the nmol/cm² range, depending on the substrate used. In addition, the Membrane has been optimized for low nonspecific binding. The Membrane is available either as a large, prenumbered, partially cut sheet (approximately 10.5 x 15.0cm; Cat.# V2861) or as a smaller, uncut sheet (approximately 7.6 x 10.9cm; Cat.# V7861). The partially cut Membrane (Cat.# V2861) allows easy separation into 96 individual squares and is designed for small-scale experiments where high binding capacity is required. The uncut sheet (Cat.# V7861) can be analyzed as a whole Membrane or may be cut into the size desired. The uncut Membrane allows for sample application using a multichannel pipettor. Both Membranes may be analyzed using phosphorimaging, autoradiography or scintillation counting to quantitate results. The Membranes have also been used successfully with chemiluminescence detection techniques. The use of fluorescence for detection of captured molecules is not recommended at this time.

The SAM²® 96 Biotin Capture Plate contains Promega's SAM²® Biotin Capture Membrane in the wells of a microfiltration plate. The 96 well plate configuration allows washes to be performed using a vacuum manifold or a commercially available plate washer. The plate is supplied with a transparent top seal and an opaque bottom seal, which allow addition of scintillation fluid for data quantitation using a microplate liquid scintillation counter.

^(a)U.S. Pat. No. 6,066,462 has been issued to Promega Corporation for quantitation of protein kinase activity.

Features:

- **Versatility:** Analysis of biotinylated substrates can be applied to a wide variety of substrate types without the need to optimize each substrate for binding to a matrix.
- **Specificity:** The combination of protein denaturant and high-salt washes minimizes nonspecific binding to the Membrane without interfering with the high affinity interaction between streptavidin and biotin.
- **High Signal-to-Noise Ratios:** The stringent washing conditions employed assist in attaining very low background counts.
- **Linear Binding:** Membrane can linearly bind biotinylated substrates up to the nmol/cm² range—allows for kinetic studies.
- **High Affinity:** Strong biotin-streptavidin binding is stable under a wide variety of wash conditions.
- **Rapid:** Biotinylated substrate binds to Membrane within 1 minute.
- **Convenient:** Compatible with enzyme assays using radioactive or chemiluminescent detection.

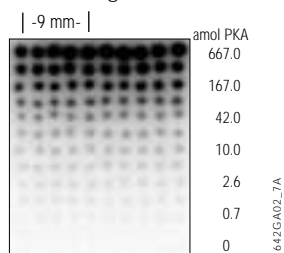
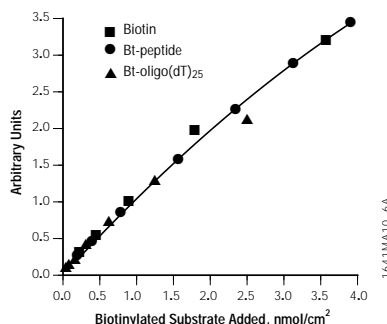


Figure 8.1. Miniaturization of kinase assay analysis using the SAM²® Biotin Capture Membrane. 0.2µl samples of 10 minute kinase reactions containing the indicated amount of cAMP Dependent Protein Kinase (PKA) were spotted on SAM²® Biotin Capture Membrane, washed and analyzed by phosphorimaging.

Figure 8.2. Binding of biotinylated substrate to the SAM²® Biotin Capture Membrane.

The indicated amount of radioactive biotin and biotinylated (Bt) peptide in 2.5M guanidine-HCl were spotted on SAM²® Biotin Capture Membrane, washed 4 times in 2M NaCl with 1% H₃PO₄ and 2 times in water, dried and counted. Radioactive biotinylated oligo(dT)₂₅ in water was spotted on SAM²® Membrane as indicated, washed 4 times in 1% SDS, 2 times in 0.1M NaCl, 4 times in 2M NaCl and 2 times in 0.1M NaCl, dried and counted.



Promega Product Citations

Orena, S.J., Torchia, A.J. and Garofalo, R.S. (2000) Inhibition of glycogen-synthase kinase 3 stimulates glycogen synthase and glucose transport by distinct mechanisms in 3T3-L1 adipocytes. *J. Biol. Chem.* **275**, 15765.

Poe, J.C. *et al.* (2000) CD22 forms a quaternary complex with SHIP, Grb2, and Shc. A pathway for regulation of B lymphocyte antigen receptor-induced calcium flux. *J. Biol. Chem.* **275**, 17420.

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Protocol

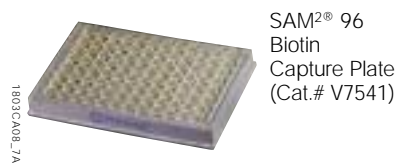
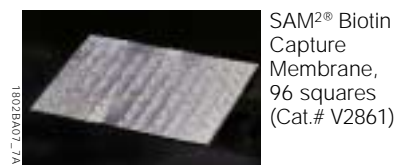
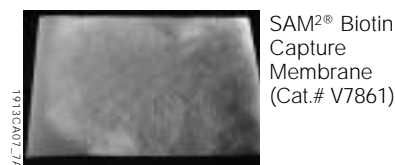
- SAM²® Biotin Capture Membrane.....**TB547**
- SAM²® 96 Biotin Capture Plate....**TB249**

Publications

Goueli, S. *et al.* (1996) SAM²® Biotin Capture Membrane and SignaTECT® Protein Kinase Assay Systems. *Promega Notes* **58**, 22.

Goueli, S. *et al.* (1997) Advances in the SAM²® Membrane Technology: High throughput biotin capture systems for use in rapid screening. *Promega Notes* **64**, 2.

Goueli, S. (2000) Protein kinases as drug targets in high-throughput systems. *Promega Notes* **75**, 24.



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Shin, H.K., Shin, Y.W. and Hong, K.W. (2000) Role of adenosine A2B receptors in vasodilation of rat pial artery and cerebral blood flow autoregulation. *Am. J. Physiol. Heart Circ. Physiol.* **278**, H339.

Campos-Neto, A. *et al.* (1998) CD40 ligand is not essential for the development of cell-mediated immunity and resistance to *Mycobacterium tuberculosis*. *J. Immunol.* **160**, 2037.

Waitumbi, J. and Warburg, A. (1998) *Phlebotomus papatasi* saliva inhibits protein phosphatase activity and nitric oxide production by murine macrophages. *Infect. Immun.* **66**, 1534.

Kim, H. *et al.* (1997) Nitric oxide modulates modulates the c-Jun N-terminal kinase/stress-activated protein kinase activity through activating c-Jun N-terminal kinase kinase. *Biochemistry* **36**, 13677.

Promega Resources

Protocol

Griess Reagent System.....**TB229**

Griess Reagent System

Product	Size	Catalog #
Griess Reagent System	1,000 assays	G2930

Description: The Griess Reagent System measures nitrite (NO²⁻), which is one of two primary stable and nonvolatile breakdown products of nitric oxide (NO). Nitric oxide is an important physiological messenger and effector molecule in many biological systems, including immunological, neuronal and cardiovascular tissues (1,2). This assay relies on a diazotization reaction that was originally described by Griess in 1879 (3). Through the years, many modifications to the original reaction have been described.

This system detects NO²⁻ in a variety of biological and experimental liquid matrices such as plasma, serum, urine and tissue culture medium. The nitrite sensitivity is dependent on the matrix (Figure 8.3). The limit of detection is 2.5µM (125pmol) nitrite (in ultrapure, deionized distilled water) using the protocol described in Technical Bulletin #TB229.

References

1. Bredt, D.S. and Snyder, S.H. (1994) *Ann. Rev. Biochem.* **63**, 175.
2. Dawson, T.M. and Dawson, V.L. (1995) *The Neuroscientist* **1**, 7.
3. Griess, P. (1879) *Chem. Ber.* **12**, 426.

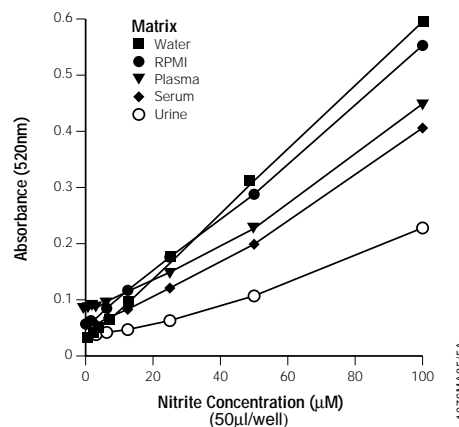


Figure 8.3. Representative Nitrite Standard reference curves in various matrices. Assays were performed as described in Technical Bulletin #TB229 using the Nitrite Standard in the following undiluted matrices: water, RPMI 1640 containing 15% serum and 5.3mg/L phenol red, bovine plasma, bovine calf serum and human urine.

Anti-pS⁴⁷³ Akt pAb

Product	Size	Catalog #
Anti-pS ⁴⁷³ Akt pAb	40µl	G7441

Description: Akt, also known as protein kinase B, is a serine/threonine, mitogen-regulated protein kinase involved in the protection of cells from apoptosis, the promotion of cell proliferation and diverse metabolic responses. Akt is activated upon binding by phospholipids and phosphorylation at residues Thr³⁰⁸ and Ser⁴⁷³ by upstream kinases such as phosphoinositide-dependent protein kinase 1 and 2 (PDK1 and PDK2). Activation of Akt results in phosphorylation and inactivation of a number of proteins involved in apoptosis, including BAD, caspase-9 and a member of the forkhead-like family of transcription factors (FKHR-L1). Currently, research into the enzymes activating Akt, as well as the downstream substrates of Akt, are under investigation.

Reference

1. Giancotti, F.G. and Rouslahti, E. (1999) *Science* **285**, 1030.

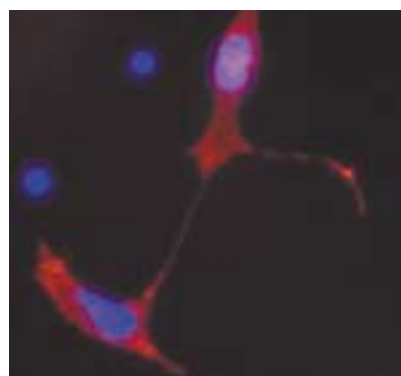


Figure 8.4. Immunocytochemical staining of Akt in embryonic rat brain cells. Embryonic (day 17) rat brain cells were collected and treated with 20ng/ml each of EGF and FGF. Anti-Akt pAb was used at a 1:50 dilution. Positive cells were visualized using donkey anti-rabbit, Cy3TM-conjugated secondary antibody (Jackson ImmunoResearch). Nuclei were stained using DAPI.

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