

REFERENCES

- Abee, T., Palmen, R., Hellingwerf, K.J., and Konings, W.N. 1990. Osmoregulation in *Rhodobacter sphaeroides*. **J. Bacteriol.** 172: 149-154.
- Barak, A.J., Beckenhauer, H.C., Junnila, M., and Tuma, D.J. 1993. Dietary betaine promotes generation of hepatic S-adenosylmethionine and protects the liver from ethanol-induced fatty infiltration. **Alcohol Clin. Exp. Res.** 17: 552-555.
- Barrett, M.C., and Dawson, A.P. 1975. Essentiality of ubiquinone for choline oxidation in rat liver mitochondria. **Biochem. J.** 148: 595-597.
- Blumwald, E., Mehlhorn, R.F., and Packer, L. 1983. Studies of osmoregulation in salt adaptation of cyanobacteria with ESR spin-probe techniques. **Proc. Natl. Acad. Sci. USA** 80: 2599-2602.
- Blumwald, E., Tel-Or, E. 1982. Osmoregulation and cell composition in salt-adaptation of *Nostoc muscorum*. **Arch. Microbiol.** 132: 168-172.
- Borowitzka, L.J., Demmerie, S., Mackay, M.A., and Norton, R.S. 1980. Carbon-13 nuclear magnetic resonance study of osmoregulation in a blue-green alga. **Sci.** 210: 650-651.
- Bradford, M. M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. **Anal. Biochem.** 72: 248-254.
- Britten, R.J., and McClure, F.T. 1962. The amino acid pool in *Escherichia coli*. **Bacteriol. Rev.** 26: 292-335.
- Brouquisse, R., Weigel, P., Rhodes, D., Yocum, C.F., and Hanson, A.D. 1989. Evidence for a ferredoxin-dependent choline monooxygenase from spinach chloroplast stroma. **Plant Physiol.** 90: 322-329.
- Brown, A.D. 1976. Microbial water stress. **Bacteriol. Rev.** 40: 803-846.

- Brown, A.D. 1978. Compatible solutes and extreme water stress in eukaryotic microorganisms. **Adv. Microb. Physiol.** 17: 181-242.
- Burton, R.F. 1983. The composition of animal cells: solutes contributing to osmotic pressure and charge balance. **Comp. Biochem. Physiol. B Comp. Biochem.** 76: 663-671.
- Cayley, S., Lewis, B.A., and Record, M.T.J. 1992. Origins of the osmoprotective properties of betaine and proline in *Escherichia coli* K-12. **J. Bacteriol.** 174: 1586-1595.
- Chi-Shui, L., and Ru-dan, W. 1986. Choline oxidation and choline dehydrogenase. **J. Prot. Chem.** 5: 193-200.
- Choquet, C.G., Ahonkhai, I., Klein, M., and Kushner, D.J. 1991. Formation and role of glycine betaine in the moderate halophile *Vibrio costicola*. **Arch Microbiol.** 155: 153-158.
- Eichenberger, W., Araki, S., and Muller, D.G. 1993. Betaine lipids and phospholipids in brown algae. **Phytochem.** 34: 1323-1333.
- Fan, L.L., and Master, B.S.S. 1974. Properties of purified kidney microsomal NADPH-cytochrome *c* reductase. **Arch. Biochem. Biophys.** 165: 665-671.
- Finkelstein, J.D., Martin, J.J., Harris, B.J., and Kyle, W.E. 1982. Regulation of the betaine content of rat liver. **Arch. Biochem. Biophys.** 218: 169-173.
- Flannery, W.L., Doetsch, R.N., and Hansen, P.A. 1952. Salt desideratum of *Vibrio costicolus*, an obligate halophilic bacterium I. Ionic replacement of sodium chloride requirement. **J. Bacteriol.** 64: 713-714.
- Forsyth, M.P., and Kushner, D.J. 1970. Nutrition and distribution of salt response in populations of moderately halophilic bacteria. **Can. J. Microbiol.** 16: 253-261.

- Galinski, E.A., and Truper, H.G. 1982. Betaine, a compatible solute in the extremely halophilic phototrophic bacterium *Ectothiorhodospira halochloris*. **FEMS Microbiol. Lett.** 13: 357-360.
- Garcia-Perez, A., and Burg, M.B. 1991. Role of organic osmolytes in adaptation of renal cells to high osmolarity. **J. Membr. Biol.** 119: 1-13.
- Garcia-Perez, A., and Burg, M.B. 1991. Renal medullary organic osmolytes. **Physiol. Rev.** 71: 1081-1115.
- Garlick, S., Oven, A., and Padan, A. 1977. Occurrence of facultative anoxygenic photosynthesis among filamentous and unicellular cyanobacteria. **J. Bacteriol.** 129: 623-629.
- Geitler, L. 1932. **Cyanophyceae**. In *Kryptogamenflora von Deutschland, Osterreich und der Schweiz*, edited by R. Kolkwitz. Akademische Verlagsgesellschaft, Leipzig. 14: 1-1196.
- Gorham J., Wyn Jones, R.G., and Mc Donnell, E.1985. Some mechanisms of salt tolerance in crop plants. **Plant Soil.** 89: 15-40.
- Hanson. A.D., Hitz, W.D. 1982. Metabolic responses of mesophytes to plant water deficits. **Annu. Rev. Plant Physiol.** 33: 163-203.
- Hanson, A.D., May, A.M., Grument, R., Bode, J., Jamieson, G.C., and Rhodes, D. 1985. Betaine synthesis in chenopods : localization in chloroplasts. **Proc. Natl. Acad. Sci. USA** 82: 3678-3682.
- Hanson, A.D.,and Scott, N.A. 1980. Betaine synthesis from radioactive precursors in attached, water-stress barley leaves. **Plant Physiol.** 66:342-348.
- Haubrich, D.R., and Gerber, N.H. 1981. Choline dehydrogenase assay, properties and inhibitors. **Biochem. Pharmacol.** 30(21): 2993-3000.

- Incharoensakdi, A., and Kum-arb U. 1998. Betaine aldehyde dehydrogenase from a halotolerant cyanobacterium *Aphanothece halophytica* : purification, properties and regulation by salinity. **J. Sci. Soc. Thailand** (in press)
- Incharoensakdi, A., Takabe, T., and Akazawa, T. 1986. Effect of betaine on enzyme activity and subunit interaction of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase from *Aphanothece halophytica*. **Plant Physiol.** 81: 1044-1049.
- Kamekura, M., Wallace, R., Hipkiss, A.R., and Kushner, D.J. 1985. Growth of *Vibrio costicola* and other moderate halophiles in a chemically defined minimal medium. **Can. J. Microbiol.** 31: 870-872.
- Kimura, T., and Singer, T. P. 1962. Choline dehydrogenase from rat liver. **Meth. Enzymol.** 5: 562-570.
- Lanfald, B., and Strom, A.R. 1986. Choline-glycine betaine pathway confers a high level of osmotic tolerance in *Escherichia coli*. **J. Bacteriol.** 165(3): 849-855.
- Le Rudulier, D., and Boullard, L. 1983. Glycine betaine, an osmotic effector in *Klebsiella pneumoniae* and other members of the *Enterobacteriaceae*. **Appl. Environ. Microbiol.** 46: 152-159.
- Le Rudulier, D., Strom, A.R., Dandekar, A.M., Smith, L.T., and Valentine, R.C. 1984. Molecular biology of osmoregulation. **Science** 224:1064-1068.
- Lerma, C., Hanson, A.D., and Rhodes, D. 1988. Oxygen-18 and deuterium labeling studies of choline oxidation by spinach and sugar beet. **Plant Physiol.** 88: 695-702.
- Lerma, C., Rich, P.J., Ju, G.C., Yang, W.J., Hanson, A.D., and Rhodes, D. 1991. Betaine deficiency in maize : complementation tests and metabolic basis. **Plant Physiol.** 95: 1113-1119.
- Lin, C.S., and Wu, R.D. 1986. Choline oxidation and choline dehydrogenase. **J. Prot. Chem.** 5: 193-200.

- Mackay, M.A., Norton, R.S., and Borowitzka, L.J. 1983. Marine blue-green algae have a unique osmoregulatory system. **Mar. Biol.** 73: 301-307.
- Mackay, M.A., Norton, R.S., and Borowitzka, L.J. 1984. Organic osmoregulatory solutes in cyanobacteria. **J. Gen. Microbiol.** 130: 2177-2191.
- MacKnight, A.D.C. 1988. Principle of cell volume regulation. **Renal Physiol. Biochem.** 3-5: 114-141.
- Manetas, Y., Petropoulou, Y., and Karabourniotis, G. 1986. Compatible solutes and their effects on phosphoenol pyruvate carboxylase of C₄-halophyte. **Plant. Cell and Environ.** 9: 145-151.
- Mc Cue, K.F., and Hanson, A.D. 1992. Salt - inducible betaine aldehyde dehydrogenase from sugar beet cDNA cloning and expression. **Plant Mol. Biol.** 18:1-11.
- Measures, J.C. 1975. Role of amino acids in osmoregulation of non-halophilic bacteria. **Nature (London)** 257: 398-400.
- Miller, D.M., Jones, J.H., Yopp, J.H., Tindall, D.R., and Schmid, W.D. 1976. Ion metabolism in a halophilic blue green alga, *Aphanothece halophytica*. **Arch. Microbiol.** 111: 145-149.
- Mohammad, F.A.A., Reed, R.H., and Stewart, W.D.P. 1983. The halophilic cyanobacterium *Synechocystis* DUN52 and its osmotic responses. **FEMS. Microbiol. Lett.** 16: 287-290.
- Nagasawa, T., Kawabata, Y., Tani, Y., and Ogata, K. 1976. Purification and characterization of betaine aldehyde dehydrogenase from *Pseudomonas aeruginosa* A-16. **Agric. Biol. Chem.** 40: 1743-1749.
- Nagasawa, T., Mori, N., Tani, T., and Okata, K. 1976. Characterization of choline dehydrogenase from *Pseudomonas aeruginosa* A-16. **Agric. Biol. Chem.** 40: 2077-2084.

- Naganishi, T., and Burg, M.B. 1989. Osmoregulatory fluxes of myo-inositol and betaine in renal cells. **Am. J. Physiol.** 257: c964-c974.
- Pan, S.M. 1988. Betaine aldehyde dehydrogenase in spinach. **Bot. Bull. Acad. Sin.** 29: 255-263.
- Perroud, B., and Le Rudulier D. 1985. Glycine betaine transport in *Escherichia coli*: osmotic modulation. **J. Bacteriol.** 161: 393-401.
- Pollard, A., and Wyn Jones, R.G. 1979. Enzymatic activities in concentrated solutions of glycinebetaine and other solutes. **Planta.** 144: 291-298.
- Randerath, K. 1963. **Thin-Layer Chromatography.** Academic press, New York and London. p-74.
- Reed, R.H., Borowitzkay, L.J., Mackay, M.A., Chudek, J.A., Foster, R., Warr, S.R.C., Moore, D.J., and Stewart, W.D.P. 1986. Organic solute accumulation in osmotically stressed cyanobacteria. **FEMS. Microbiol. Rev.** 39: 51-56.
- Reed, R.H., Chudek, J.A., Foster, R., and Stewart, W.D.P. 1984. Osmotic adjustment in cyanobacteria from hypersaline environments. **Arch. Microbiol.** 138: 333-337.
- Reed, R.H., Richardson, D.L., Warr, S.R.C., and Stewart, W.D.P. 1984. Carbohydrate accumulation and osmotic stress in cyanobacteria. **J. Gen. Microbiol.** 130:1-4.
- Reed, R.H., Stewart, W.D.P. 1983. Physiological responses of *Rivulariaatra* to salinity: osmotic adjustment in hyposaline media. **New. Phytol.** 95: 595-603.
- Reed, R.H., Warr, S.R.C., Richardson, D.L., Moore, D.L. Stewart, W.D.P. 1984. Multiphasic osmotic adjustment in a euryhaline cyanobacterium. **FEMS. Microbiol. Lett.** 28: 225-229.
- Rendina, G., and Singer, T.P. 1959. Studies on choline dehydrogenase. **J. Biol. Chem.** 234:1605-1610.

- Robinson, S.P., and Jones, G.P. 1986. Accumulation of glycinebetaine in chloroplast provides osmotic adjustment during salt stress. **Aust. J. Plant Physiol.** 13: 659-668.
- Roller, S.D., and Anagnostopoulos, G.D. 1982. Accumulation of carbohydrate by *Escherichia coli* B/r/1 during growth at low water activity. **Appl. Microbiol.** 52: 425-434.
- Rothschild, H.A., and Barron, E.S.G. 1954. The oxidation of betaine aldehyde by betaine aldehyde dehydrogenase. **J. Biol. Chem.** 209: 511-523.
- Russell, R., and Scopes, R.K. 1994. Use of hydrophobic chromatography for purification of the membrane-located choline dehydrogenase from a *Pseudomonas* strain. **Bioseparation.** 4: 279-284.
- Shkedy-Vinkler, C., and Avi-Dor, Y. 1975. Betaine-induced stimulation of respiration at high osmolarities in halotolerant bacterium. **Biochem. J.** 150: 219-226.
- Smith, L.T., Pocard, J.A., Bernard, T., and Le Rudulier, D. 1988. Osmotic control of glycine betaine biosynthesis and degradation in *Rhizobium meliloti*. **J. Bacteriol.** 170: 3142-3149.
- Stewart, W.D.P. 1983. Natural environments-challenges to microbial success and survival. **Symp. Soc. Gen. Microbiol.** 34: 1-35.
- Streumer-Svobodova, Z., and Drahota, Z. 1977. The development of oxidative enzymes in rat liver mitochondria. **Physiol. Bohemoslov.** 26: 525-534.
- Storey, R., Wyn Jones, R.G. 1977. Quaternary ammonium compounds in plants in relation to salt resistance. **Phytochem.** 16: 447-453.
- Strom, A.R., Le Rudulier, D., Jakowec, M.W., Bunnell, R.C., and Valentine, R.C. 1983. Osmoregulatory (Osm) genes and osmoprotective compounds. *In Genetic engineering of plants*, edited by T. Kosuge, C. Meredith, and A. Hollaender. Plenum Publishing Corp., New York. P. 39-59.

- Strom, A.R., Falkenberg, P., and Lanfald, B. 1986. Genetics of osmoregulation in *Escherichia coli* : Uptake and biosynthesis of organic osmolytes. **FEMS Microbiol. Rev.** 39: 79-86.
- Thongekkaw, J. 1995. **Production and purification of phycocyanin from *Aphanothece halophytica***. Thesis of master of science programme of biotechnology, Chulalongkorn university. 1-112.
- Tsuge, H., Nakano, Y., Onishi, H., Futamura, Y., and Ohashi, K. 1980. A novel purification and some properties of rat liver mitochondrial choline dehydrogenase. **Biochim. Biophys. Acta.** 614: 274-284.
- Von Hippel, P.H., and Schleich, T. 1969. The effects of neutral salts on the structure and conformational stability of macromolecules in solution. *In* **Structure and stability biological micromolecules**, edited by S.N. Timash-off Marcel Dekker, New York 2: 417-574.
- Weigel, P., Weretilnyk, E.A., and Hanson, A.D. 1986. Betaine aldehyde oxidation by spinach chloroplasts. **Plant Physiol.** 82: 753-759.
- Weretilnyk, E.A., and Hanson, A.D. 1989. Betaine aldehyde dehydrogenase from spinach leaves: Purification, *in vitro* translation of the Mrna, and regulation by salinity. **Arch. Biochem. Biophys.**, 27: 56-63.
- Wilken, D.R. 1970. Estimation of choline and betaine aldehyde dehydrogenase activities in rat liver mitochondria by three independent methods. **Anal. Biochem.** 36: 323-331.
- Wilken, D.R., McMacken, M.L., and Rodriguez, A. 1970. Choline and betaine aldehyde oxidation by rat liver mitochondria. **Biochim. Biophys. Acta.** 216: 305-317.
- Williams, J.N., and Screenivasan, A. 1953. Preparation of soluble choline dehydrogenase from liver mitochondria. **J. Biol. Chem.** 203: 899-906.

- Wirthensohn, G., and Guder, W.G. 1982. Studies on renal choline metabolism and phosphatidylcholine synthesis. **Biochem. of kidney function.**, edited by F. Morel. Amsterdam: Elsevier. 119-128.
- Wutipraditkul, N., Kum-arb, U., and Incharoensakdi, 1999 A. Factors affecting the accumulation of glycine betaine in a halophytic cyanobacterium, *Aphanothece halophytica*. **J. Sci. Res. Chula. Univ.**(in press)
- Wyn Jones, R.G., Gorham, J. 1983. Osmoregulation. *In Encyclopedia of plant physiology*, edited by Lange O.L., Nobel, P.S., Osmond, C.B., and Ziegler, H. Springer, Berlin Heidelberg New York. 12: 35-58.
- Wyn Jones, R.G., Storey, R., Leigh, R.A., Ahmad, N., and Pollard, A. 1977. A hypothesis on cytoplasmic osmoregulation. *In Regulation of cell membrane activities in higher plants*, edited by E. Marre and C. Ciferri. Elsevier, North Holland, Amsterdam. P. 121-136.
- Wyn Jones, R.G., Storey, R. 1981. Betaines. *In The physiology and biochemistry of drought resistance in plants*, edited by L.G. Paleg and D. Aspinall. Academic Press Australia, Sydney, Australia. P.171-204.
- Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D., and Somero, G.N. 1982. Living with water stress: evolution of osmolyte systems. **Science** 217: 1214-1222.
- Yopp.J.H., Miller, D.M., and Tindall, D.R. 1978. Regulation of intracellular water potential in the halophilic blue-green alga *Aphanothece halophytica* (*Chroococcales*). *In Energetics and structure of halophilic microorganisms*, edited by Caplan, S.R., and Ginzburg, M. Elsevier, Amsterdam. P. 619-624.
- Zeisel, S.H., and Blusztajn, J.K. 1994. Choline and human nutrition. **Ann. Rev. Nutr.** 14: 269-296.

Zhang, J, Blusztajn, J.K., and Zeisel, S.H. 1992. Measurement of the formation of betaine aldehyde and betaine in rat liver mitochondria by a high pressure liquid chromatography radioenzymatic assay. **Biochim. Biophys. Acta.** 117: 333-339.



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APPENDIX

APPENDIX 1

Turk Island Salt Solution + modified BG₁₁ medium contained the following components:

1. Preparation of Turk Island Salt Solution

Stock Solution A : KCl	33.3 g
MgCl ₂ .6H ₂ O	275.0 g
CaCl ₂ .2H ₂ O	73.3 g

and made up to 5 litres with distilled water

Stock Solution B: MgSO ₄ .7H ₂ O	347.0 g
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and then made up to 5 litres with disstilled water

To make Turk Island Salt Solution, 500ml of Stock Solution A was added to 500 ml of Stock Solution B. To this mixture 140.8 g of NaCl was added and the final volume was made to 5 litres with distilled water.

2. Composition of modified BG₁₁ medium (BG₁₁ medium + NaNO₃ Solution)

NaNO ₃	(75 g/500 ml)	50 ml
KH ₂ PO ₄	(8 g/200 ml)	5 ml
MgSO ₄ .7H ₂ O	(15 g/200ml)	5 ml
CaCl ₂ .2H ₂ O	(7.2 g/200 ml)	5 ml
Na ₂ CO ₃	(4 g/200 ml)	5 ml
Citric acid	(1.2 g/200 ml)	5 ml
EDTA.Na ₂	(0.2 g/200 ml)	5 ml

$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (1.2 g/200 ml) 5 ml

*Trace element A₆ Solution + Co 5 ml

*Trace element A₆ Solution + Co contained the following component in gram per litre H_3PO_4 : 2.86 ; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$: 0.2 ; $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$: 0.08 ; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$; 1.81 ; $\text{Na}_2\text{MnO}_4 \cdot 2\text{H}_2\text{O}$: 0.39 ; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$: 0.049

Culture medium of *Aphanothece halophytica* was prepare by adding all solution of item 2 at indicated volume to 5 litres of Turk Island Salt Solution and the pH was adjusted to 7.6 by slowly adding 2 M NaOH. The medium was sterilized by autoclaving at 15 lb/in² for 15 minutes.



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APPENDIX 2**Dragendorff's reagent****Stock solution****Solution A**

bismuth subnitrate 17 g

tartaric acid 200 g

adjust volume to 800 ml with distilled water

Stock solution B

potassium iodide 160 g

adjust volume to 400ml with distilled water

mixed solution A with B

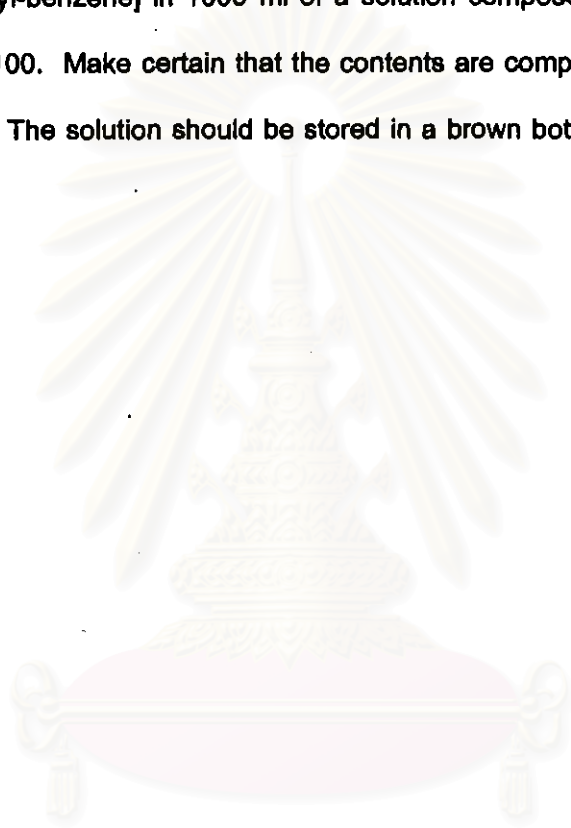
For use, 100 g tartaric acid is dissolved in 50 ml of this mixture (solution A and B) and 250 ml water

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APPENDIX 3

Scintillation fluid (1000 ml) as follows :

Dissolve 5.5 g PPO (2,5-diphenyloxazole) and 0.1 g POPOP [1,4-bis (5-phenyloxazole-2-yl-benzene)] in 1000 ml of a solution composed of 667 ml Toluene and 333 ml Triton X-100. Make certain that the contents are completely dissolved before the solution is used. The solution should be stored in a brown bottle in a cool dark place.



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APPENDIX 4**Preparation of polyacrylamide gel electrophoresis:****1. Stock reagent****Solution A (30% Acrylamide, 0.8% bis stock solution)**

acrylamide 30.3 g

bis-acrylamide 0.8 g

adjust volume to 100 ml with distilled water

Solution B

For native-PAGE

Tris 18.2 g

adjust pH to 8.8 with 1 M HCl and adjust volume to 100 ml with distilled

water

Solution C

For native-PAGE

Tris 6.0 g

adjust pH to 6.8 with 1 M HCl and adjust volume to 100 ml with distilled

water

Electrophoresis buffer

For native-PAGE

Tris 3.0 g

Glycine 14.4 g

adjust pH to 8.3 with 1 M HCl and adjust volume to 1000 ml with distilled

water

2. Non-denaturing PAGE

7.5% Separating gel

Solution A 2.5 ml

Solution B 2.5 ml

distilled water 5.0 ml

TEMED 10.0 μ l10%(w/v) $(\text{NH}_4)_2\text{S}_2\text{O}_8$ 50.0 μ l5% Stacking gel

Solution A 1.3 ml

Solution C 2.0 ml

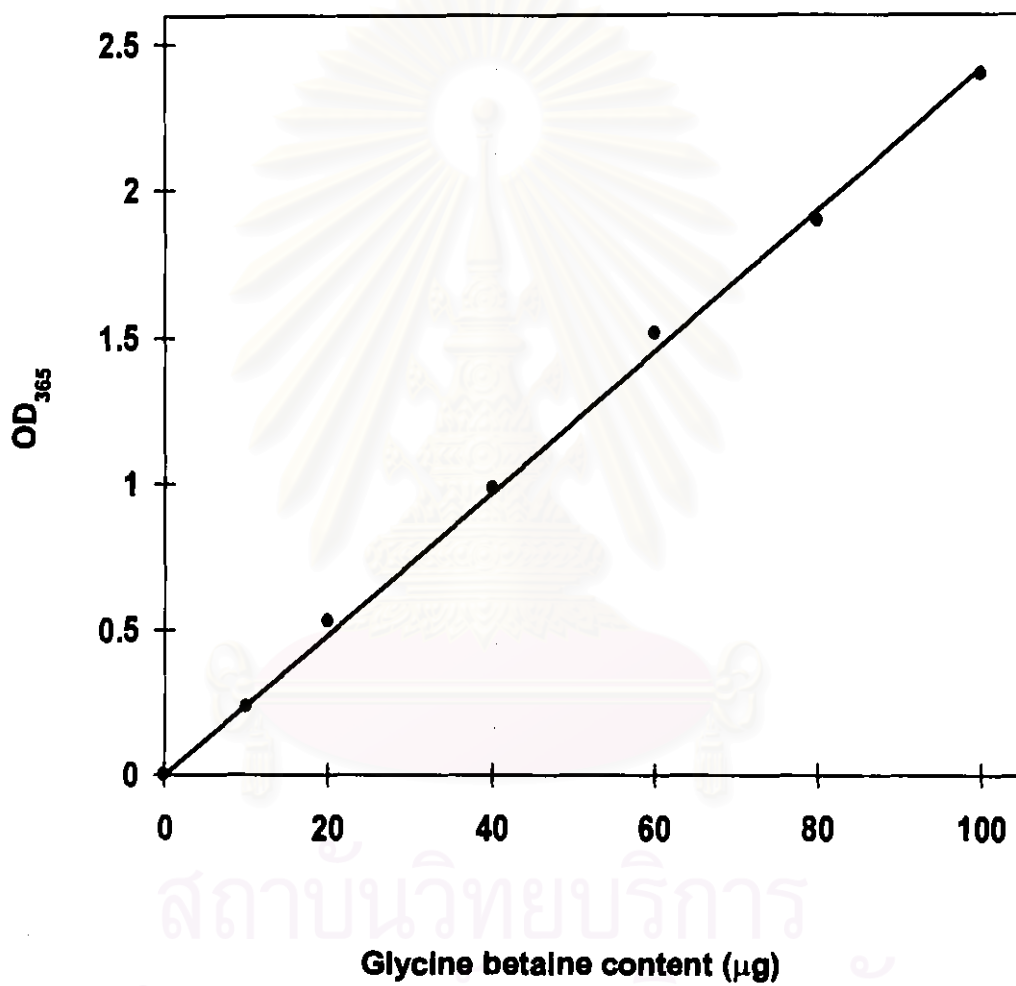
distilled water 4.6 ml

TEMED 10.0 μ l10%(w/v) $(\text{NH}_4)_2\text{S}_2\text{O}_8$ 60.0 μ lSample buffer

1.25 M Tris-HCl pH 6.8: glycerol: distilled water (1:2:2, v/v) were added with trace amount of bromo-phenol blue.

APPENDIX 5

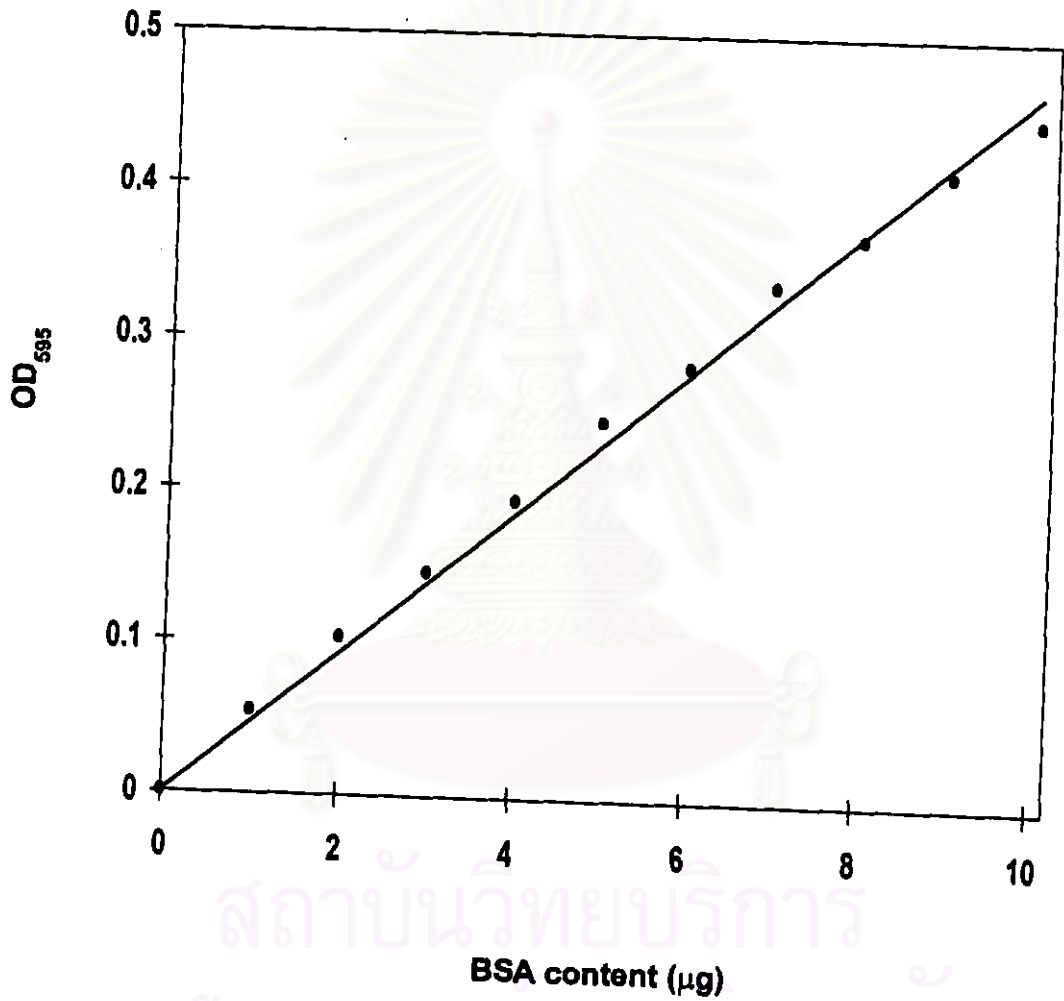
Standard curve of glycine betaine content by Trilodide assay



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APPENDIX 6

Standard curve of BSA by Bradford assay



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BIOGRAPHY

Miss Nuchanat Wutipraditkul was born on February 10, 1970 In Bangkok, Thailand. She graduated with a Bachelor of Science Degree in Radiological Technology from Faculty of Medical Technology, Mahidol University, Thailand in 1992 and studied for a Master Degree in Biochemistry program since 1995



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