REFERENCES

Bagshawe, K.D., Searle, F., and Springer, G. 1989. Tumor site activation of cytotoxic agents. Brit. J. Cancer 59.

Baldwin, R.W. 1985. Monoclonal antibody targeting of anti- cancer agents. Eur. J. Cancer Clin. Oncol. 21: 1281-1285.

Beasley, R.P. 1982. Hepatitis B virus as the etiologic agent in HCC-epidermiologic considerations. Hepatology 2: 215-265.

Berge, J.C.S. 1991. Monoclonal antibodie for Therapy of human malignant tumors. Eur. J. Surg. Suppl. 561: 59-64.

Berman, C. 1951. Primary carcinoma of the liver. London: H.K. Lewis.

Blakey, D. C., Wawrzynczak, E. J., Wallace, P. M., and Thrope, P. E. 1988. Antibody toxin conjugates: a perspective. Prog. Allergy 45: 50-90.

Boring, C.C., Tong, T. 1992. Cancer Statistics, 1992. Cancer 42: 19-36.

Britton, K.E., Mather, S.J., and Granowska, M. 1991. Radiolabelled monoclonal antibodies in oncology III radioimmunotherapy. Nuclear Medicine Communications 12: 333- 347.

Brodeur, B.R., Tsang, P., and Larase, Y. 1984. Parameters affecting ascites tumor formation in mice and MAb production. J. Immunol. Methods 71: 265.

Budillon, A., Tagliaferri, P., Caraglia, M., et al. 1991. Upregulation of epidermal growth factor recepter induced by α interferon in human epidermoid cancer cells. Cancer Res. 51: 1294-1299.

Byers, V.S., and Baldwin, R.W. 1988. Therapeutic strategies with monoclonal antibodies and immunoconjugates. Immunology 65: 329-335.

Carlson, R.I., Porath, E.B., Shouval, D., Strauss, W., Isselbacher, K.J., and Wands, J.R. 1985. Antigenic characterization of human hepatocellular carcinoma. J. Clin. Invest. 76: 40-51.

Catane, R., and Longo, D. L. 1988. Monoclonal antibodies for cancer therapy. Isr. J. Med. Sci. 24: 471-476.

Cheung, N.K.V., Lazarus, H., Miraldi, F.D., et al. 1987. Glanglioside GD 2 specific monoclonal antibody 3F8: A phase I study in patients with neuroblastoma and malignant melanoma. J. Clin. Oncol. 5: 1430-1440.

Chlebowski, R., Tong, M., Weissman, J., et al. 1984. Hepatocellular carcinoma-diagnostic and prognosis features in North American patients. Cancer 53: 2701-2706.

Coog, G.G., and Moosa, B. 1985. Hepatocellular carcinoma: One of the world's most common malignancies. Am. J. Med. 233: 705-708.

_____, Mozaffari, P., Van Rensberg, S. 1985. Cancer of the liver. Br.Med. Bull. 40: 342-345.

Corvalan, J.A.F., Smith, W., and Gore, V.A. 1988. Tumor therapy with vinca alkaloids targeted by a hybrid - hybrid monoclonal antibody recognising both CEA and vinca alkaloids. Int. J. cancer 2: 22-25.

Denardo, S.J., Denardo, G.L., Macey, D.J., et al. 1988. Successful radioimmunotherapy in patients with lymphoma; results of dose ftactionation of I-131 LYM-1 monoclonal antibody. J. Nucl. Med. 29: 847.

Di Bisceglie, A.M., and Hoofnagle, J.H. 1987. Heptitis B virus infection and HCC: Etiologic relationships and clinical implications. In updates: Cancer: Principles and practice of oncology. Philadelphia: JB Lippincott. 10: 1-10.

_____, Rustgi, V.K., Hoofnagle, J.H., et al. 1988. Hepatocellular carcinoma. Ann. Intern. Med. 108: 390-401.

Dillman, R.O. 1989. Monoclonal antibodies for treating cancer. Ann. Int. Med. 111: 592-603.

_____Beauregard, J.C., Halpern, S.E., and Clutter, M. 1986. Toxicity and side effects associated with intravenous infusions of murine monoclonal antibodies. J. Biol. Resp. Med. 5: 73-84.

_____Shawler, D.L., Dillman, J.B., and Royston, I. 1984. Therapy of chronic lymphocytic leukemia and cutaneous T-cell lymphoma with T 101 monoclonal antibody. J. Clin. Oncol. 2: 881-891.

Dunk, A.A., Novick, D., and Thomas, H.C. 1987. Natural killer cell activity in HCC : in vitro and in vivo response to interferon. Scan-J. Gastroenterol. 22: 1245-1250.

Dvorak, H.F., Nagy, J.A., and Dvorak, A.M. 1991. Structure of solid tumirs and their vasculature: Implications for therapy with monoclonal antibodies. Cancer Cells 3: 77-85.

Eggle, H. 1910. Uber das primare carcinom der leber. Beitr Z Path Anat Z allg Path. 30: 506-604.

Embleton, M. J. 1986. Targeting of anti-cancer therapeutic agents by monoclonal antibodies. Biochem. Soc. Trans. 14: 393-395.

Epenetos, A.A., Courtenay - Luck, N., Pickering, D., Hooker, G., et al. 1985. Antibody guided irradiation of brain glioma by arterial infusion of radioactive monoclonal antibody against epidermal growth factor recepter and blood group A antigen. Br. Med. J. 290: 1463-1466.

Ey, P.L., Prowse, S.J., and Jenkins, C.R. 1978. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulin from mouse serum using protein A- sepharose. Immunochemistry 15: 429-436.

FitzGerald, D., and Pastan, I. 1989. Targeted toxin therapy for the treatment of cancer. J. Natl. Cancer Inst. 81: 1455-1463.

Foon, F.A., and Morgan, A.C. 1985. Monoclonal antibody therapy of human cancer. Massachusetts: Martinus Nijhoff Publishing.

Foon, K.A., Schroff, P.W.,Bunn, P.A., et al. 1984. Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia. Blood 64: 1085-1094.

_____1989. Biological response modifiers : The new immunotherapy. Cancer Res. 49: 1621-1639.

Fukuda, Y., Imai, K., Miura, K., et al. 1988. A monoclonal antibody to the carbohydrate chain on human hepatocellular carcinoma- associated antigen which suppressed tumor growth in nude mice. Cancer- Immunol-Immunother. 27: 26-32.

Fu-Sun, Y., and Kong-Nien, S. 1986. Epidermiology and early diagnosis of primary liver cancer in China. Adv. Cancer Res. 47: 297-329.

Glenn, M.J., and Stevenson, G.T. 1985. Derivatives of anti-idiotype antibodies in the treatment of B-cell lymphoma. Adv.Exp.Med.Biol. 186: 843-853.

Gresham, H.D., Mcgarr, J.A., Shackelford, P.G., and Brown, E.J. 1988. Studies on the molecular mechanisms of human Fc receptor-mediated phagocytosis. J. Clin. Invest. 82: 1192-1201.

Guadagnu, F., Roselli, M., Amato, T., Cosinelli, M., et al. 1991. Clinical evaluation of serum tumor- associated glycoprotein-72 as a novel tumor marker for colorectal cancer patient. J. Surg. Oncol. Supple. 2: 16-20.

Halver, J.E. 1965. In mycotoxin in foodstuffs (edited by G.N. Wogan) Cambridge, Massachusetts. pp.209.

Harlow, E., and Lane, D. 1988. Antibodies: A laboratory manual. Cold Spring Harbor Laboratory.

Hata, K., Theil, D.H.V., Herberman, R.B., and Whiteside, T.L. 1991. Natural killer activity of human liver-derived lymphocytes in various liver diseases. Hepatology 14: 495-503.

Hellstorm, I., Beaumier, P. L., and Hellstorrn, K.E. 1986. Antitumor effects of L6 an IgG2a antibodies with most human carcinomas. Proc. Natl. Acad. Sci. USA83: 7059-7063.

_____Garrigues, U., Lavie, E., and Hellstorm, K.E. 1988. Antibody - mediated killing of human tumor cells by attached effector cells. Cancer Res. 48: 624-627.

Hellstorm, K.E., and Hellstorm, I. 1989. Oncogene-associated tumor antigens as targets for immunotherapy. FASEB J. 3: 1715-1722.

Herlyn, D., and Koprowski, H. 1982. IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells. Proc. Natl. Acad. Sci. USA 79: 4761-4765.

_____Lubeck, M., Steplewski, Z., and Koprowski, H. 1985. In monoclonal antibodies and cancer therapy, UCLA symphosia on Molecular and cellular biology. New York: Liss. 27: 165-172.

_____Powe, J., Ross, A. H.,Herlyn, M., and Koprowski, H. 1985. Inhibition of human tumor growth by IgG2a monoclonal antibodies correlates with antibody density on tumor cells. The Journal of Immunology 134: 1300-1304.

Hiraiwa, N., Fukuda, Y., Imura, H., et al. 1990. Accumulation of highly acidic sulfated glycosphingolipids in human hepatocellular carcinoma defined by a series of monoclonal antibodies. Cancer Res. 50: 2917-2928.

Hobbs, K.E.F. 1987. Liver transplantation: a review. J. Hepatol. 4: 148-153.

Hoofnagle, J.H., Shafritz, D.A., and Popper, H. 1987. Chronic type B hepatitis and the " healthy" HBsAg carrier state. Hepatology 7: 758-763.

Hoogenraad, N. , Helman, J., and Hoogeraad, J. 1983. The effects of pre - injection of mice with pristane on ascites tumor formation and MAb production. J. Immunol. Methods. 61: 317.

Houghton, A.N., Mintzer, D., Cordon-Cardo, C., et al. 1985. Mouse monoclonal IgG3 antibody detection DGd 3 ganglioside: aphase I trial in patients with malignant melanoma. Proc. Natl. Acad. Sci. USA 82: 1242-1246.

Hu, C-P., Han, S-H., Lui, W-Y., et al. 1986. Monoclonal antibodies against antigen expressed on human hepatocellular carcinoma cells. Hepatology 6: 1396-1402.

Ihde, D., Matthews, M., Markuch, R., and et al. 1985. Prognosis factors in patient with hepatocellular carcinoma receiving systemic chemotherapy-identification of two groups of patients with prospects for prolonged survival. Am. J. Med. 78: 399-406.

Johnstone, A., and Thrope, R. 1987. Immunochemistry in Practice. 2nd ed. London: Blackwell Scientific Publications.

Kenshead, J.T., Jones, D.H., Lashford, L., et al. 1986. 131-I coupled to monoclonal antibodies as therapeutic agents for neuroectodermally derived tumors: fact or fiction. Cancer Drug Deliv. 3: 25-43.

Koda, K., and Glassy, M.C. 1990. In Vitro immunuzation for the production of human monoclonal antibody. Hum. Antibod. Hybridoma 1: 15-22.

Kohlor, G., and Milstein, C. 1975. Continuous culture of fused cells secreting antibody of predefined specificity. Nature 256: 495-497.

Kosmas, C., Kalofonos, H.P., and Epenetos, A.A. 1990. Radiolabelled monoclonal antibodies in tumor diagnosis and therapy. Develop. Biol. Standard. 71: 93-102.

Laohathai, K., and Bhamarapravati, N. 1985. Culturing human hepatocellular carcinoma: Simple and reliable method. Am. J. Pathol. 118: 203-208.

Laohathai, K., Capone, P., Daiken, K., and Chu, T. M. 1985. Monoclonal antibody to primary hepatocellular carcinoma. FASAB Federation proceedings 44: 531.

Larrick, J.W., and Bourla, J.M. 1986. J.Biol. Res. Modif. 5: 379.

_____, and Buck, D.W. 1984. Bio. Technique 14: 6.

Lembersky, B. C. 1991. Imolication of current therapeutic approaches in colorectal cancer for other gastrointestinal malignancies. Semin-Oncol. 18: 39-46.

Levy, P.C., Shaw, G.M., and LoBurlio, A.F. 1979. Human monocyte,lymphocyte, and granulocyte antibody-dependent cell-mediated cytotoxicity toward tumor cells. J. Immuno. 123: 594-599.

Lieberman, M.D., Sigal, R. K., Noel, N., et al. 1991. Natural killer cell stimulatory factor (NKSF) augments natural kill cell and antibody- dependent tumoricidal response against colon catcinoma cell lines. J. Surg. Res. 50: 410-415.

Linsell, A. 1987. Primary liver cancer : Epidermiology and etiology. In Wanebo HJ(ed): Hepatic and biliary cancer. New York: Marcel Dekker.

Linsell, C.A., and Peers, F.G. 1977. Trans R Soc Trop Med Hyg. 71: 471.

Livraghi, T.A., Bolondi, L.S., et al. 1988. Small hepatocellular carcinoma: percuneous alcohol injection-results in 23 patients. Radiology 168: 313-317.

Lutwick, L.I. 1979. Relationship between aflatoxin, hepatitis virus, and hepatocellular carcinoma. Lancet 1: 755-757.

Maki, R.G., Old, L.J., and Srivastava, P.K. 1990. Human homoologue of murine tumor rejection antigen gp96: 5'-regulatory and coding regions and relationship to stress-induced proteins. Proc. Natl. Acad. Sci. USA 87: 5658-5662.

Maraj, R., Kew, M.C., and Hyslop, R.J. 1988. Resectability rate of hepatocellular carcinoma in rural southern Africans. Br. J. Surg. 75: 335-338.

Markham, N. A., James, R. O. et al. 1986. Primary HCC localised by a radiolabelled monoclonal antibody. J. Hepatol. 2: 25-31.

Matsui, M., Nakanishi, T., Nogushi, T., Imai, K., et al. 1985. Suppression of human melanoma growth in nude mice injected with anti-high-molecular-weigth melanoma- associated antigen monoclonal antibody 225.28s conjugated to purothionin. Jpn. J. Cancer Res.(Gann) 76: 119.

Meeker, T.C., Lower, J., Maloney, D.G., et al. 1985. A clinical trail of anti-idiotype therapy for B-cell malignancy. Blood 65: 1345-1363.

Motoo, Y., Hill, N.O., Mahmoudi, M., and Osther, K. 1986. Anti tumor effect of human tumor necrosis factor on human hepatoma cells PLC/PRF/5. Japan J. Exp. Med. 56: 151-154.

Munz, D.L., Alaw, A., Koprowski, H., Herlyn, D. 1986. Improved radioimaging of human tumor xenografts by a mixture of monoclonal antibody F(ab') fragments. J. Nucl. Med. 27: 1739-1745.

Nadler, L.M., Stashenko, P., Hardy, R., et al. 1980. Scrotherapy of a patient with a monoclonal antibody directed against a human lymphoma - associated antigen. Cancer Res. 40: 3147-3154.

Nakashima, T., and Kojiro, M. 1987. HCC: An attlas of its pathology H. Popper Springer-Verlag.

Novelle, J. R., Markham, N. I., and Hobbs, K. E. F. 1989. New hope in irresectable hepatoma. Hepato- gastroenterol. 36: 258-261.

Ohzu, K., Hasegawa, K., Yamauchi, K., and Obata, H. 1990. Multiplicity of newly established monoclonal antibodies against hepatocellular carcinomas. J. Gastroenterol. Hepatology 5: 601-6

Okuda, K. 1986. Early Recognition of hepatocellular carcinoma. Hepatology 6: 729-738.

1986. Primary liver cancer. Dig. Dis. Sci. 31: 133S-46S.

_____, Obata, H., Nakajima, Y., et al. 1984. Prognosis of primary hepatocellular carcinoma. Hepatology 4: 35-145.

_____, Ohtsuki, T., Obata, H., and et al. 1985. Natural history of hepatocellular carcinoma and prognosis in relation to treatment: study of 850 patients. Cancer 56: 918-928.

Olson, S. 1986. "Biotechnology: An industry comes of age" Washington, D.C.: National Academy Press.

Oldham, R.K., Foon, K.A., Morgan, A.C., et al. Monoclonal antibody therapy of malignant melanoma: in vivo localization in cutaneous metastasis after inteavenous administration. J. Clin. Oncol. 2: 1235-1246.

Order, S.E., Ettirger, D.S., and Liebel, S.A. 1988. Cyclic radiolabelled 131-I anti ferritin in multimodality therapy of hepatocellular carcinoma. J. Immunol. 13: 45-51.

_____, Stillwagon, G.B., Klein, J.L., and et al. Iodine 131- anti- ferritin: a new treatment modality in hepatoma: a radiation therapy oncology group study. J.Clin. Oncol. 3: 1573-1582.

Ortaldo, J.R., Woodhouse, C., Morgan, A.C., Herbermann, R.B.,vCheresh, D.A., Reisfeld, R. 1987. Analysis of effector cells in human antibody - dependent cellular cytotoxicity with murine monoclonal antibodies. J. Immunol. 138: 3566-3572.

Osborn, M. 1990. Antibody in diagnosis and therapy. Seminars in cancer biology Philadelphia: Saunders Scientific Publications Company.

Public Health statistic A.D. 1990. Division of Health statistics, Office of the permanent secretary Ministry of Public Health.

Ravikumar, T.S., Kane, R., Cady, B., et al. 1987. Hepatic cryosurgery with intraolerative ultrasound monitoring for metastatic colon carcinoma. Arch. Surg. 122: 403-409.

Reisfeld, R.A., Schulz, G., and Cheresh, D.A. 1985. In monoclonal antibodies and cancer therapy, UCLA symphosia on molecular and cellular biology. New York: Liss. 27: 173-191.

Robinson, W.D., Miller, R.H., Klote, L., et al. 1984. Viral hepatitis and liver disease. New York: Grune and Strattion.

Sato, G. H., and Sato, J. D. 1989. Growth factor receptor monoclonal antibodies and cancer immunotherapy. J. Natl. Cancer Inst. 81: 1600-1601.

Schlom, J. 1986. Basic principles and applications of monoclonal antibodies in the management of carcinomas. Cancer Res. 46: 3225-3228.

Schulz, G., Bumol, T.F., and Reisfeld, R.A. 1983. MAb-directed effector cells selectively lyse human melanoma cells in vitro and in vivo. Proc. Natl. Acad. Sci. USA 80: 5407-5411.

Sears, H.F., Herlyn, D., Staplewski, Z., and Koprowski, H. 1985. Phase II clinical trial of a murine monoclonal antibody cytotoxic for gastrointestinal adenocarcinoma. Cancer Res. 45: 5910-5913.

Sell, S., and Reisfeld, R.A. 1985. Monoclonal antibodies in cancer New Jersey: Humana Press.

Silverberg, E. 1988. Cancer statistics 1987. Cancer 38: 5-22.

Sharkey, R.M., Henessy, M., Siegel, J.A., and Goldenberg, D.M. 1990. Biodistribution and radiation dose estimates for yt-trium and iodine-labelled monoclonal antibody IgG and fragments in nude mice bearing human colonic tumor xenografts. Cancer Res. 50: 2330-2336.

Sheu, J.C., Sung, J.L., Chen, D.S., et al. 1985. Early detection of hepatocellular carcinoma by real- time ultrasonography. Cancer 56: 660-666.

Shimizu, J., Zou, J-P., Ikegame, K. et.al. 1991. evidence for the functional binding in vivo of tumor rejection antigens to antigen-presenting cells in tumor-bearing hosts. J. Immuno. 146: 1708-1714.

Shimotohno, K. 1993. Viral hepatitis and hepatocellular carcinoma: A new challange for cancer prevention. "The Eleventh Asian Pacific Cancer Conference" November 16-19.

Shinagawa, T., Ohto, M., Kimura, K., et al. 1984. Diagnostic and clinical features of small hepatocellular carcinoma with emphasis on the utility of real-time ultrasonography: A study of 51 patients. Gastroenterology 86: 496-502.

Shouval, D., Eilat, D., Carlson, R.I., Adler, R., Livni, N., Wands, J.R. 1985. Human hepatoma-associated cell surface antigen: Identification and characterization by means of monoclonal antibody. Hepatology 5: 347-356.

Smans, K.A., Hoylaertes, M.F., Hendrickx, H.F., et al. 1991. Tumor cell lysis by in-situ - activated human peripheral- blood mononuclear cells. Int. J. Cancer 47: 431- 438.

Takahashi, H., Ozturk, M., Wilson, B., et al. 1989. In vivo expression of two novel tumor-associated antigens and their use in immunolocalization of human hepatocellular carcinoma. Hepatology 9: 625-634.

Takayasu, K., Shima, Y., Muramatsu, Y., et al. 1987. Hepatocellular carcinoma: treatment with intra-arterial iodized oil with and without chemotherapeutic agents. Radiology 162: 345-351.

Tang, .Z.Y. 1985. Subclinical HCC New York: Springer Verlag.

Tao, Q.M. 1985. Epidermiology of persistent infection with hepatitis B virus in chronic liver diseases. In: hepatocellular carcinoma in Asia. Kobe, Japan: Kobe University School of Medicine. 3-6.

Tian-P-Y., Zhang-ML., Huang-J., Yu-B., and Zhen-Y-S. 1989. Specific binding and internalization of anti-CCT2 MAb and bleomycin Ab conjugate in human leukemia cells. Yao-Hsueh-Hsueh-Pao. 24: 16-21.

Tjandra, J.J., Pietersz, G.A., Smyth, M.J., and McKenzie, I.F.C. 1988. Role of monoclonal antibodies in the therapy of solid tumors. Aust. N.Z.Y. Surg. 58: 843-849.

Thekerngpol, K., Khawcharoenporn, V., Mangkalannond, K., Sindhavananda, K. and Stitnimamkarn, T. 1983. Hepatocellular carcinoma: An electron microscopic study of 52 cases. J. Med. Ass. Thai 66(2): 735-745.

Thompson, K.M. 1988. Immunol. Today 9: 113.

Vatamasapt, V., Martin, N., Sriplung, H., et al. 1993. Cancer in Thailand(1988-1991) IARC Technical Report NO.16 Lyon.

Viranuvatti, V. 1984. Primary liver cancer in Thailand. Tropical gastroenterology 5: 75-81.

Vincent, T., Hellman, S., and Rosenberg, S.A. 1989. Cancer Principles and practice of oncology. 3rd ed. Philadelphia: J.B. Lippincott Company.

Warunee Dansithong. 1994. Effects of interferon on hepatoma cell lines observed by electron microscopy. Master's thesis. Chulalongkorn University.

Zalutsky, M.R. 1989. Antibodies in radiodiagnosis and therapy. Florida: CRC Press,Inc.

Zaman, S.N., Melia, W.N., Johson, R.D., Portmann, B.C., Johnson, P.J., and Williums, R. 1985. Risk factors in development of hepatocellular carcinoma in cirrhosis: Prospective study of 613 patients. Lancet 1: 1357-1360.

Zhang, Y. J., Tang, Z.Y., Xie, H., Yao, Z., Yuan, A.N., Zhao, H.Y., Lu, J.Z. 1991. Imaging and therapy of human HCC with radiolabeled monoclonal antibody. J. Exp. Clin. Cancer Res. 10: 243-252.

Zola, H. 1987. Monoclonal antibodies: A manual of techniques. CRC Press, Inc.

APPENDIX

BUFFERS AND REAGENTS

BUFFERS:

Phosphate buffer stock solution (PB), pH 7.4

0.2 M $NaH_2PO_4 . 2H_2O$, pH 4.5	31.202 gm/l
0.2 M $NaHPO_4 . 7H_2O$, pH 9.1	53.614 gm/l

Add NaH_2PO_4 to $Na_2 HPO_4$ dropwise to adjust the pH to 7.2 - 8.0 .

Phosphate buffer saline (PBS), pH 7.4

0.2 M PB, pH 7.2 - 7.4 stock solution	50 ml
NaCl	8.76 gm
Distilled water to make	1000 ml

0.1 M Citrate buffer , pH 3.0 - 7.0 (for an affinity chromatography)

0.1 M Citric acid ($C_6H_8O_7 . 1H_2O$)	21.014 g/l
0.1 M $Na_2HPO_4.7H_2O$	26.807 g/l

For pH 5.0 is approximately a 50:50 mixture of citric acid to phosphate , below pH5.0 : titrate pH of citric acid with phosphate and above pH 5.0 : titrate pH of phosphate with citric acid . Autoclave before add thimerosal to 0.01 % as preservative .

1.0 M Tris - hydrochloric acid buffer , pH 8.5 and 9.0

1 M $C_4H_{11}NO_3$	121.1 g/l
1 M HCl	

For pH 8.5 prepare by mix 25 ml of 1 M Tris with 7.2 ml 1 M HCl.

For pH 9.0 prepare by mix 25 ml of 1 M Tris with 2.5 ml 1 M HCl .

Autoclave before add thimerosal (0.01 %).

0.2 M Cacodylate buffer , pH 7.4

Sodium carcodylate	42.8 gm
Distilled water to make	1000 ml

Dissolve Sodium carcodylate in distilled water and mix well . Adjust pH to 7.4 with 0.1 N HCl

0.1 M Cacodylate buffer , pH 7.4

0.2 M cacodylate buffer, pH 7.4	1 part
Distilled water	1 part

Veronal acetate buffer

stocking solution:

NaCl	3.4 %
Sodium acetate	1.94 %
Sodium barbital	2.94 %

working solution:

0.22 M Veronal acetate buffer , pH 7.4

veronal acetate stocking solution	5 ml
Distilled water	13 ml
1 M $CaCl_2$	0.25 ml

Add 0.1 N HCL to adjust pH.

0.11 M Veronal acetate buffer, pH 7.4

0.22 M veronal acetate buffer	1 part
Distilled water	1 part

Gold Buffer (for dilute Protein-A gold conjugate)

Tris-HCL	6.61 gm
Tris base	0.97 gm
NaCl	8.77 gm
Distilled water	800 ml

Adjust pH to 7.4 with 0.1 N HCL and bright volume to 1000 ml with distilled water.

REAGENTS:

1.5 % Agar

Agar	0.15 gm
Dissolve water to make	10 ml

Dissolve agar with distilled water. Heat until boiling and cool at room temperature until the temperature become 37° C before use.

100% Ammonium sulfate $(NH_3)_2SO_4$

Ammonium Sulfate	100 gm
Distilled water	100 ml

Dissolve this amount of ammonium sulfate at 50°C until the solvent is cleared , stand overnight at room temperature , adjust the pH to 7.2 with dilute ammonia solution .

0.5 % BSA

Bovine serum albumin	0.5 gm
0.01 M PBS wirh 0.01 % thimerosal	100 ml

Stirring the two components until clearly. Filter with filter paper before store at 4°c .

1 % Glutaraldehyde

50 % Glutaraldehyde	0.2 ml
0.2 M Phosphate buffer, pH 7.4	5.0 ml
Distilled waterto make 10 ml	

OPD Substrate (for ELISA Assay)

OPD	0.008 gm
Phosphate citrate buffer, pH 5.0	20 ml
H_2O_2 30 %	8 µl

Dissolve OPD in phosphate citrate buffer pH 5.0 and mix well . Immediately use after add 30 % H_2O_2 (reducing agent).

Protease inhibitor (stock solution)

Benzamidine	10 mM
Epsilon amino carproic acid	10 mM
Phenyl methyl sulfonyl Pluoride	10 mM
50 % Ethanol	

Dissolve all the described above in 50 % Ethanol. The stock solution was diluted to 1:10 or 1:100. Store at 4°C and protect from light .

Reduced Osmium

Stocking solution:

1) 4 % Osmium Tetroxide

OsO_4	1 gm
Distilled water	25 ml

2) 2 % $K_4Fe(CN)_6$

$K_4Fe(CN)_6$	0.1 gm
0.3 M cacodylate buffer, pH 7.4	5 ml
CaCl2 (2.5 mM)	0.027 gm

Working solution:

Mix equal amount of 4 % osmium tetroxide and 2 % $K_4Fe(CN)_6$ (preparation before use)

Spurr resin (Embedding media)

VCD (4 -vinycyclohexene dioxide)	10 gm
DER resin	6 gm
NSA (Nonenyl Succinic Anhydride)	26 gm
DMAE (Dimethylaminoethanol)	0.4 gm

Mix all together immediately before use and after add the DMAE.

0.4 % Trypan blue

Trypan blue (vital stain)	400 mg
Distilled water	90 mg

NaCl	810 mg
K_2HPO_4	60 mg
Methyl p- hydroxy benzoate	50 mg

Dissolve all of the components in distilled water. Mixture was heated to boiling. After the mixture cooled and the pH was adjust to 7.2-7.3 with 1 N NaOH (approximate 8 drops). Finally, adjust to final volume of 100 ml with distilled water .

0.05% Trypsin

Stosking solution:

1) 0.02 % EDTA (Versene)

NaCl	8 gm
KCl	0.2 gm
Na_2HPO_4	1.15 gm
KH_2PO4	0.2 gm
EDTA	0.2 gm
Distilled water	1 L
Phenol red	0.01 gm

Dissolve all chemicals in distilled water.

2) PBS (CMF) , pH 7.4 (CA,Mg free)

NaCl	8 gm
KCl	0,2 gm
Na_2HPO_4	1.15 gm
KH_2PO_4	0.2 gm
Distilled water	1 L
Phenol red	0.01 gm

Dissolve all chemical in distilled water and adjust pH to 7.4 with 1 N NaOH .

3) 2.5 % Trypsin

PBS (CMF)	100 ml
Trypsin	2.5 gm

Dissolve trypsin in PBS(CMF) and mix well. Sterile with 0.22 μm microfilter before store at -20°c . Thawing before use.

working solution:

2.5 % Trypsin	2 ml
EDTA	49 ml
PBS(CMF)	49 ml

Mix all solution into complete solution. For sterilization by filter with 0.22 μm milipore.

Staining solution (for EM thin section)

1) Lead citrate

Lead nitrate	0.3325 gm
Sodium citrate	0.44 gm
Distilled water	7.5 ml
NaOH 1 N	2 ml
Distilled water	2.5 ml

Dissolve lead nitrate and sodium citrate in distilled water 7.5 ml , mix well and stand at room temperature 30 minutes . Shaking until it become milky then add 1 N NaOH 2 ml and mix will . When it is clear , add 2.5 ml of distilled water . It must be filter before use .

2) 0.5 % Uranyl acetate

Uranyl acetate	0.5 gm
30 % EtOH to make	100 ml

Dissolve and mix gentle, filter through 0.22 μm milipore. This solution is light sensitive. It is possible to be stored frozen in small aliquotes.

1 %Toluidine blue stain (for EM thick section)

Toluidine blue	1 gm
Sodium borate , pH 12	1 gm
Distilled water to make	100 ml

Dissolve sodium borate in distilled water to make 100 ml . Then, add toluidine blue and mix well.

Culture media:

RPMI media

RPMI 1640	10.41 gm/L
Sterile water	1000 ml
$NaHCO_3$	2 gm
Pen-Strep stock solution	10 ml

Dissolve and shake until all component are completely dissolved. For sterilization, filter through 0.22 μm milipore.

RPMI hybrid media

RPMI 1640	10.41 gm/L
Sterile water	1000 ml
$NaHCO_3$	2 gm

Pen-Strep stock solution	10 ml
L-glutamine	0.1 gm
D-glucose	2 gm
Pyruvic acid	0.11 gm

Dissolve and shake until all component are completely dissolved. For sterilization, filter through 0.22 μm milipore.

Pennicillin-Streptomycin (stock solution)

Pennicillin G sodium	1,000,000 units
Streptomycin	1 gm
Distilled water	100 ml

Dissolve all of the components in distilled water and mix well.

AUTHOR BIOBLIOGRAPHY

Miss Siripen Thongpassano was born on Febuary 1st, 1969 in Nakhonsithammarat, Thailand. She received her Bachelor of Science in Nursing and Midwifery (first class honors) in 1991 from the Faculty of Nursing, Prince of Songkla University, Songkla, Thailand. She has enrolled Chulalongkorn University in the Master of Science Program with a specialization in academic year 1992. During her study, she was supported by grant from the National Science and Technology Development Agency (NSTDA).