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ภาคผนวก ก.

น้ำยา สารเคมี และวัสดุอุปกรณ์ สำหรับการทำ HLA TYPING

น้ำยาและสารเคมี

Calcium carbonate ($CaCO_3$) (Merck, Darmstadt, West Germany)

Disodium hydrogen phosphate (Na_2HPO_4) (Merck, Darmstadt, West Germany)

Ficoll-Paque solution (Pharmacia, Uppsala, Sweden)

Formaldehyde 37% (HCHO) (Merck,Darmstadt, West Germany)

Glycerine ($CH_2OHCHOHCH_2OH$) (Farmitalia Carlo Erba, Milano, Italy)

Hanks' balanced salt solution (HBSS) (Gibco, N.Y., U.S.A)

Heparin 1,000 unit/ml (Novo, Copenhagen, Denmark)

Hepes(N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid)(Sigma,MO,U.S.A)

Latex (Difco, Detroit, Michigan, U.S.A.)

Mineral oil, Light No.M-3516 (Sigma, Missouri, U.S.A.)

Penicillin-Streptomycin 1,000 unit/ml (Gibco, New York, U.S.A.)

Phosphate buffer salt (PBS) (Flow Laboratories, Irvine, U.K.)

Potassium chloride (KCl) (Merck, Darmstadt, West Germany)

Potassium dihydrogen phosphate (KH_2PO_4) (Merck, Darmstadt, West Germany)

RPMI-1640 (Gibco, N.Y., U.S.A.)

Sodium chloride (NaCl) (Merck, Darmstadt, West Germany)

Sodium hydrogen carbonate ($NaHCO_3$) (Merck, Darmstadt, West Germany)

Thrombin 1,000 unit/ml (Parke-Davis, New Jersey, U.S.A.)

ซีรั่มและแอนติบอดีย์

Anti Human Immunoglobulin-FITC (Wellcome, Dartford, England)

HLA Antisera (Biotest, Frankfurt, West Germany)

Fetal bovine serum (Flow Lab, North Ryde, Australia)

Rabbit complement for HLA-A,B,C (Pel-Freez, Wisconsin, U.S.A.)

Rabbit complement for HLA-DR (Pel-Freez, Wisconsin, U.S.A.)

สี

Eosin-Y ($C_{20}H_6Br_4Na_2O_5$) (Merck, Darmstadt, West Germany)

Trypanblue ($C_{34}H_{24}N_6Na_4O_{14}S_4$) (Merck, Darmstadt, West Germany)

วัสดุวิทยาศาสตร์

Filter paper, Whatman No. 1 (Whatman, Maidstone, England)

Microtest plate (Nunc, Roskilde, Denmark)

Membrane filters (Whatman, Maidstone, England)

Nylon wool (Fenwal, Illinois, U.S.A.)

Scalp vein (Nipro, Osaka, Japan)

Syringe (disposable) (Terumo, Tokyo, Japan)

เครื่องแก้ว

Beaker (Pyrex, corning, N.Y., U.S.A.)

Cylinder (Pyrex, corning, N.Y., U.S.A.)

Erlenmeyer flask (Kimax, Ohio, U.S.A.)

Pasteur pipette (Kimble, Ohio, U.S.A.)

Petri dish (Pyrex, corning, N.Y., U.S.A.)

Serological pipette (Witeg, West Gemany)

หลอดทดลองและวัสดุพลาสติก

Centrifuge tube, 50 ml (Nunc, Roskilde, Denmark)

Centrifuge tube, 10 ml (Nunc, Roskilde, Denmark)

Cryotube (Nunc, Roskilde, Denmark)

Fisher's tube (Fisher Scientific, U.S.A.)

Microcentrifuge tube (Beckman, California, U.S.A.)

Pipette tip (Finntip, Helsinki, Finland)

Plastic pipette (Sarstedt, West Germany)

Planing slide (Lux, Miles Laboratories, Illinois, U.S.A.)

อุปกรณ์วิทยาศาสตร์

Automatic pipette (Gilson, Lyon, France)

Dispenser (Hamilton, Bonaduz, Switzerland)

Forcep (Pro-med, West Germany)

Hemacytometer (AO, N.Y., U.S.A.)

Jet pipette (York Instruments, California, U.S.A.)

Microsyringe (Hamilton, Bonaduz, Switzerland)

Pincet (Pro-med, West Germany)

เครื่องมือวิทยาศาสตร์

Deep freezer (Revco, North Carolina, U.S.A.)

Fisher's centrifuge (Fisher Scientific, U.S.A.)

Incubator (Memmert, Schwabach, West Germany)

Microscopes

- Bright field (Olympus, Tokyo, Japan)
- Fluorescent (Olympus, Tokyo, Japan)
- Inverted phase contrast (Zeiss, Oberkochen, West Germany)

Refrigerated centrifuge (Kokusan, Tokyo, Japan)

Suction pump (Thomus, Wisconsin, U.S.A.)

Water bath (Memmert, Schwabach, West Gemany)

ภาคผนวก ข.

น้ำยา สารเคมี และวัสดุอุปกรณ์ สำหรับการทำ C2 TYPING

น้ำยาและสารเคมี

Acrylamide (LKB, Bromma, Sweden)

Ammonium persulphate (Merck, Darmstadt, West Germany)

Ampholine (LKB, Bromma, Sweden)

Bis acrylamide (LKB, Bromma, Sweden)

4-chloro-1-naphthol (Sigma, MO, U.S.A.)

Ethylene diamine tetra acetic acid (EDTA) (Merck, Darmstadt, West Germany)

Gelatin (Merck, Darmstadt, West Germany)

Hydrogen peroxide 30% (H_2O_2) (Merck, Darmstadt, West Germany)

Isoelectric focusing marker (Sigma, Missouri, U.S.A.)

Methanol (CH_3OH) (Merck, Darmstadt, West Germany)

Ortho-phosphoric acid 85% (H_3PO_4) (Merck, Darmstadt, West Germany)

Sodium chloride (NaCl) (Merck, Darmstadt, West Germany)

Sodium hydroxide (NaOH) (Merck, Darmstadt, West Germany)

Sucrose ($C_{12}H_{22}O_{11}$) (Merck, Darmstadt, West Germany)

Trizma base (Sigma, MO, U.S.A.)

Taurine (2-Aminoethanesulfonic acid) (Sigma, MO, U.S.A.)

TEMED (N,N,N',N'-tetramethylethylenediamine) (Sigma, MO, U.S.A.)

Tween 20 (Sigma, MO, U.S.A.)

HP

แอนติบอดีย์

Rabbit anti-human C2 (Wako, Osaka, Japan)

Goat anti-rabbit IgG peroxidase (Hy-Clone, Utah, U.S.A.)

วัสดุวิทยาศาสตร์

Chromatography paper (3 MM. Chr) (Whatman, Maidstone, England)

Glass plate (12.5 cm. x 25 cm. x 0.2 cm.) (Bangkok, Thailand)

Nitrocellulose filter membrane (0.45 um, BA85) (Schleicher and Schuell, U.S.A.)

Spacer (0.5 mm. polyvinyl chloride sheet) (Japan)

อุปกรณ์เครื่องมือ

Clamping set (LKB Bromma, Haake, West Germany)

Cooling plate (LKB Bromma, Haake, West Germany)

Cooling pump (LKB Bromma, Haake, West Germany)

Power supply (LKB Bromma, Haake, West Germany)

ภาคผนวก ค.

การเตรียมน้ำยา
สำหรับการทำ HLA TYPING

การเตรียมน้ำยาสำหรับการแยกเซลล์ลิมโฟซัยท์

1. Hanks' balanced salt solution (HBSS) (pH 7.2 - 7.4)

HBSS	9.8	กรัม
$NaHCO_3$	0.35	กรัม
Hepes	2.86	กรัม
น้ำกลั่น	980	มล.

ปรับ pH ให้ได้ 7.2 ด้วย 5 N NaOH หรือ 5 N HCl เติมน้ำกลั่นจนครบ 1,000 มล. ทำให้ปราศจากเชื้อโดยกรองผ่าน filter membrane ขนาด 0.45 µm. เก็บที่อุณหภูมิ 2° - 8° ซ

2. RPMI - 1640 medium (pH 7.2 - 7.4)

RPMI - 1640	10.4	กรัม
$NaHCO_3$	2.0	กรัม
Penicillin - Streptomycin (10,000 ยูนิต/มล.)	2.0	มล.
Hepes	5.7	กรัม
น้ำกลั่น	980	มล.

ปรับ pH ให้ได้ 7.0 ด้วย5N NaOH และ 5N HCl เติมน้ำกลั่นจนครบ 1,000 มล. ทำให้ปราศจากเชื้อโดยกรองผ่าน filter membrane ขนาด 0.45 µm. เก็บที่อุณหภูมิ 2°-8° ซ

การเตรียมน้ำยาสำหรับตรวจสอบคุณภาพของเซลล์

1. Mounting media (50% Glycerol)

Glycerol	2.0 มล.
PBS (pH 7.4)	2.0 มล.

2. Phosphate buffer saline (PBS) 0.15M (pH 7.4)

NaCl	8.0 กรัม
KCl	0.2 กรัม
Na_2HPO_4	1.15 กรัม
KH_2PO_4	0.2 กรัม
น้ำกลั่น เติมให้ปริมาตรครบ	1,000 มล.

ปรับ pH ให้ได้ 7.4 ด้วย 5N NaOH หรือ 5N HCl

3. 0.4% Trypanblue

3.1 stock solution (1% Trypanblue)

trypanblue	0.1 กรัม
น้ำกลั่น	10.0 มล.

กรองผ่านกระดาษ Whatman No. 1 เก็บที่อุณหภูมิ 2°- 8° ซ

3.2 working solution (0.4.% Trypanblue)

1% Trypanblue	4.0 มล.
น้ำเกลือ	6.0 มล.

กรองผ่านกระดาษ Whatman No. 1 เก็บอุณหภูมิ 4°ซ

การเตรียมน้ำยาสำหรับการทดสอบ MICROLYMPHOCYTOTOXICITY TEST

1. 5% Eosin - y

Eosin - y	5.0 กรัม
น้ำเกลือเติมให้ครบปริมาตร	100 มล.

กรองผ่านกระดาษ Whatman No. 1

2. 40% Neutral formalin

37% Formaldehyde solution	40 มล.
น้ำกลั่น	60 มล.

ผสมให้เข้ากันแล้วใส่ $CaCO_3$ จำนวนมากเกินพอ ตั้งทิ้งไว้ 1 คืน ดูดเฉพาะส่วนใสกรองผ่านกระดาษกรอง Whatman No.1 ปรับ pH ให้ได้ 7.2-7.4 โดยใช้ 1N NaOH หรือ 1N HCl

ภาคผนวก ง.

การเตรียมน้ำยา
สำหรับการทำ C2 TYPING

การเตรียม POLYACRYLAMIDE GEL SOLUTION

29.1% Acrylamide	2.7 มล.
0.9% Bisacrylamide	2.7 มล.
Taurine	0.4 กรัม
Sucrose	2.0 กรัม
เติมน้ำกลั่นให้ได้ปริมาตร	16.0 มล.

อุ่นที่อุณหภูมิห้อง 37°ซ ประมาณ 20 นาที

Ampholine pH 3.5 - 9.5	100 µl.
Ampholine pH 5 - 7	900 µl.
20% Ammonium persulphate	30 µl.
TEMED	5 µl.

หมายเหตุ Ammonium persulphate และ TEMED เป็นสารที่เร่งให้เกิด polymerization จะผสมใน gel solution เมื่อพร้อมที่จะเทเจลเท่านั้น

การเตรียม BUFFER SOLUTION สำหรับการทำ ISOELECTRIC FOCUSING

1. Anode buffer solution (1M H_3PO_4)

H_3PO_4	11.53	มล.
เติมน้ำกลั่นจนครบปริมาตร	100	มล.

2. Cathode buffer solution (1M NaOH)

NaOH	4	กรัม
เติมน้ำกลั่นจนครบริมาตร	100	มล.

การเตรียมน้ำยาสำหรับ PROTEIN BLOT

1. 1M Tris - HCl (pH 7.2)

Trizma base	12.11	กรัม
น้ำกลั่น	85	มล.

ปรับ pH ให้ได้ 7.2 ด้วย conc.HCl แล้วเติมน้ำกลั่นจนครบปริมาตร 100 มล.

2. 1M NaCl

NaCl	29.2	กรัม
เติมน้ำกลั่นจนครบปริมาตร	500	มล.

เก็บที่อุณหภูมิ 2°- 8°ซ

3. 0.1M EDTA (Ethylenediamine tetra acetic acid)

EDTA	3.72	กรัม
น้ำกลั่น	95	มล.

ปรับ pH ให้ได้ 7.4 ด้วย 5N NaOH เติมน้ำกลั่นจนครบปริมาตร 100 มล. เก็บที่อุณหภูมิ 2° - 8°ซ

4. Transfer buffer for protein blotting

1M Tris - HCl (pH 7.2)	10	มล.
0.1M EDTA (pH 7.4)	20	มล.
1M NaCl	50	มล.
เติมน้ำกลั่นจนครบปริมาตร	1,000	มล.

การเตรียมน้ำยาสำหรับ IMMUNOSTAINING

1. Washing buffer (PBS + 0.1% Tween)

Tween 20	1	มล.
PBS (pH 7.4)	999	มล.

เก็บที่อุณหภูมิ 2° - 8°ซ

หมายเหตุ การเตรียม PBS ดูรายละเอียดในภาคผนวก ค.

2. Blocking buffer (PBS + 0.1% Tween + 0.1% Gelatin)

gelatin	0.1 กรัม
PBS + 0.1% Tween	70 มล.

ละลายที่อุณหภูมิ 50°ซ เมื่อละลายแล้วเติมPBS + 0.1% Tweenจนปริมาตรครบ 100 มล.

3. Substrate solution

4-chloro-1-naphthol	30 มก.
methanol	10 มล.
1M Tris-HCl (pH 7.2)	50 มล.
30% H_2O_2	10 ul

ข้อควรระวัง :

- ให้ละลาย 4-chloro-1-naphthol ใน methanol ก่อน
- 30% H_2O_2 ให้ผสมลงใน solution เมื่อพร้อมที่จะใช้แล้วเท่านั้น
- substrate solution ให้เตรียมใหม่ทุกครั้งที่จะใช้ และเตรียมใส่ขวดสีชา เนื่องจาก substrate นี้ไวต่อแสง

ประวัติผู้เขียน

นางสาวอรทัย กังวาลชิรธาดา เกิดเมื่อวันที่ 6 มกราคม 2499 ที่จังหวัดกรุงเทพมหานครฯ สำเร็จการศึกษาปริญญาวิทยาศาสตรบัณฑิต (เทคนิคการแพทย์) จากมหาวิทยาลัยมหิดล เมื่อปี พ.ศ. 2521 และเข้ารับราชการที่แผนกธนาคารเลือด โรงพยาบาลศิริราช ในตำแหน่งนักวิทยาศาสตร์เป็นเวลา 4 ปี จากนั้นจึงได้เปลี่ยนมาทำงานด้าน Tissue typing ที่ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ตั้งแต่ปี พ.ศ. 2525 จนถึงปัจจุบัน