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ภาคผนวก

น้ำยาค้างว และการเตรียม

1. น้ำยาสําหรับเพาะเลี้ยงเซลล์

1.1 fetal bovine serum (FBS)

inactivate ที่ 56° ซ นาน 30 นาที เก็บที่ -20° ซ

1.2 HEPES buffer 1 M, pH 7.3

HEPES 235.3 gm

Deionized distilled water 990 ml

ปรับ pH ด้วย 5 N NaOH ให้ได้ 7.3

เติมนํ้าจนครบ 1000 ml

ทำให้ปราศจากเชื้อโดยการกรอง เก็บที่ 4° ซ

1.3 7.5 % Sodium bicarbonate

NaHCO₃ 7.5 gm

Deionized distilled water 100 ml

ทำให้ปราศจากเชื้อโดยการ autoclave ที่ความดัน 15 ปอนด์

เป็นเวลา 15 นาที และเก็บที่ 4° ซ

1.4 อาหารเลี้ยงเซลล์ (culture media)

1.4.1 Growth media (GM)

MEM 10x 10 ml

Heat-inactivated FBS 10 ml

Penicillin, 20,000 U/ml	0.5 ml
Streptomycin, 20,000 ug/ml	0.5 ml
Amphotericin B, 200 ug/ml	1.0 ml
7.5 % NaHCO ₃	2.0 ml
HEPES, 1 M	2.0 ml
Deionized distilled water จนครบ	100 ml

เก็บที่ 4° C

1.4.2 Maintenance medium (MM).

MEM 10x	10 ml
Heat-inactivated FBS	2.0 ml
Penicillin, 20,000 u/ml	0.5 ml
Streptomycin, 20,000 ug/ml	0.5 ml
Amphotericin B, 200 ug/ml	1.0 ml
Gentamicin, 2,500 ug/ml	1.0 ml
7.5 % NaHCO ₃	2.0 ml
HEPES, 1 M	2.0 ml
Deionized distilled water จนครบ	100 ml

เก็บที่ 4° C

1.4.3 Transport media

Heat-inactivated FBS	2.0 ml
Penicillin, 100,000 u/ml	1.0 ml
Streptomycin, 100,000 ug/ml	1.0 ml
Gentamicin, 2,500 ug/ml	1.0 ml
PBS pH 7.4 จนครบ	100 ml

เก็บที่ 4° C

1.5 ยาปฏิชีวนะ

1.5.1 Gentamicin, 2,500 ug/ml

Gentamicin sulfate 80 mg 2 ml

Deionized distilled water 30 ml

แบ่งใส่หลอดเล็ก ๆ เก็บที่ -20° ซ

1.5.2 Penicillin G, 20,000 u/ml

Sodium penicillin G 1,000,000 u 1 ml

Deionized distilled water 49 ml

แบ่งใส่หลอดเล็ก ๆ เก็บที่ -20° ซ

1.5.3 Streptomycin, 20,000 ug/ml

Streptomycin 1 gm

Deionized distilled water 50 ml

แบ่งใส่หลอดเล็ก ๆ เก็บที่ -20° ซ

1.5.4 Fungizone, 200 ug/ml

Amphotericin B 50 mg/vial

Deionized distilled water 250 ml

แบ่งใส่หลอดเล็ก ๆ เก็บที่ -20° ซ

1.6 Phosphate buffer saline, 0.15 M, pH 7.4

NaCl 8.0 gm

KCl 0.2 gm

Na₂PO₄ (anhydrous) 1.15 gmKH₂PO₄ 0.2 gm

Deionized distilled water 1,000 ml

หาให้ปราศจากเชื้อโดย autoclave ที่ 15 ปอนด์ นาน 15 นาที

เก็บที่ 4° ซ

1.7 Trypsin versene solution (TV-solution) 10x

Trypsin	5.0 gm
EDTA	2.0 gm
NaCl	9.0 gm
Deionized distilled water	1,000 ml

หาให้ปราศจากเชื้อโดยการกรอง เก็บที่ -20° C

เมื่อจะใช้ให้เจือจาง 10 เท่า ด้วยน้ำกลั่นที่ปราศจากเชื้อ

2. น้ยาสสำหรับวิธี indirect immunofluorescent

2.1 Phosphate buffer saline, 0.15 M, pH 7.4

NaCl	8.0 gm
KCl	2.0 gm
Na_2HPO_4 (anhydrous)	1.15 gm
KH_2PO_4	2.0 gm
Distilled water	1000 ml

ปรับ pH เป็น 7.4 เก็บที่ 4° C

2.2 Counter stain

Evans blue	0.01 gm
Distilled water	300 ml

เก็บที่ 4° C

2.3 Buffer glycerol mounting medium

Glycerol	90 ml
PBS, pH 7.4	10 ml

เก็บที่อุณหภูมิห้อง



3. วัสดุสำหรับวิธี avidin-biotin-ELISA

3.1 Coating buffer, pH 9.6

Na_2CO_3	1.59 gm.
NaHCO_3	2.93 gm.
Distilled water	1000 ml.

ปรับ pH เป็น 9.6 ด้วย 1 M NaOH เก็บที่ 4° C

3.2 Washing buffer pH 7.2

$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	0.345 gm.
Na_2HPO_4	1.063 gm.
NaCl	29.220 gm.
Tween 20	1.0 ml.
Distilled water	1,000 ml.

pH 7.2 + 0.3

3.3 Dilution buffer สำหรับ samples และ avidin-peroxidase

Washing buffer	100 ml.
BSA	5 gm.

เก็บที่ 4° C

3.4 Dilution buffer สำหรับเจือจาง biotinylated anti-RSV.

Washing buffer	11.9 ml.
Normal rabbit immunoglobulin	0.6 ml.

เก็บที่ 4° C

3.5 0.1 M Citric acid-phosphate buffer pH 5.0

Citric acid. H ₂ O	7.30 gm
Na ₂ HPO ₄	9.47 gm
Distilled water	1000 ml
pH 5.0 เก็บที่ 4° C	

3.6 OPD-Enzyme substrate pH 5.0

OPD	8 mg
0.1 M Citric acid-phosphate buffer	12 ml
30% H ₂ O ₂	5 ul

ใช้สารละลายนี้เก็บในที่มืดที่ 4° C ใน 2 ช.ม.

3.7 Stopping solution, 4 N H₂SO₄

H ₂ SO ₄ Conc.	112 ml
Distilled water ให้ครบ	1,000 ml

. เก็บที่อุณหภูมิห้อง

3.5 0.1 M Citric acid-phosphate buffer pH 5.0

Citric acid. H ₂ O	7.30 gm
Na ₂ HPO ₄	9.47 gm
Distilled water	1000 ml
pH 5.0 เก็บที่ 4° C	

3.6 OPD-Enzyme substrate pH 5.0

OPD	8 mg
0.1 M Citric acid-phosphate buffer	12 ml
30% H ₂ O ₂	5 ul

ได้สารสีส้มเก็บในที่มืดใช้ภายใน 2 ช.ม.

3.7 Stopping solution, 4 N H₂SO₄

H ₂ SO ₄ Conc.	112 ml
Distilled water ให้ครบ	1,000 ml

เก็บที่อุณหภูมิห้อง

ประวัติผู้เขียน



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