

เอกสารอ้างอิง



1. ประวิทย์ ชูเกียรติขันธ์ "สถานภาพโรคพิษสุนัขบ้าในประเทศไทยและปัญหาการควบคุมป้องกัน" ของกองวิทยาศาสตร์ สภาวิชาการไทย ปี พ.ศ.2531 พิมพ์ใน รายงานการประชุมสัมมนาโรคพิษสุนัขบ้าในประเทศไทย การปรับเปลี่ยนเพื่ออนาคต, หน้า 17-26, 2531
2. ประวิทย์ ชูเกียรติขันธ์ "ระบาดวิทยาโรคพิษสุนัขบ้าในประเทศไทย" การป้องกันและควบคุมโรคพิษสุนัขบ้าในประเทศไทย กรุงเทพมหานคร: สำนักพิมพ์เมดิคัลมีเดีย, หน้า 30-32, 2527
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ภาคผนวก ก

สารเคมี อุปกรณ์ และเครื่องมือต่าง ๆ

1. สารเคมี

Guanidine (Aminomethanamidine) thiocyanate salt

($\text{CH}_3\text{N}_3\text{-HSCN}$) (Sigma, MO., USA)

Sarcosyl ($\text{C}_{15}\text{H}_{30}\text{N NaO}_2$) (Sigma, MO., USA)

2-mercaptoethanol (Sigma, MO., USA)

Sodium acetate (CH_3COONa) (Sigma, MO., USA)

phenol; ultra pure phenol redistilled nucleic acid
grade (BRL, USA)

Chloroform (CHCl_3) (Merck, Germany)

Isoamyl alcohol ($\text{C}_5\text{H}_{11}\text{OH}$) (Farmitalia
carlo erba S.P.A., Milano)

Tri-sodium citrate ($\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$) (Merck, Germany)

Bromophenol blue ($\text{C}_{19}\text{H}_{10}\text{Br}_4\text{O}_5\text{S}$) (Sigma, MO., USA)

Xylene cyanol FF ($\text{C}_{25}\text{H}_{27}\text{N}_2\text{O}_6\text{S}_2\text{Na}$) (Sigma, MO., USA)

Sucrose ($C_{12}H_{22}O_{11}$) (Sigma, MO., USA)
Diethylpyrocarbonate ($C_6H_{10}O_5$) (Sigma, MO., USA)
Isopropanol
Absolute ethanol (C_2H_5OH) (E. Merck, Darmstadt, Germany)
Sodium chloride (NaCl) (BDH, England)
Sodium hydroxide (NaOH) (BDH, England)
Agarose; ultra pure electrophoresis grade (Gibco BRL, USA)
EDTA (Ethylenediamine tetraacetic acid (Sigma, MO., USA)
Ethidium bromide ($C_{21}H_{20}N_3Br$) (Sigma, MO., USA)
Trisma base ($C_4H_{11}NO_3$) (Sigma, MO., USA)
Deoxynucleotide triphosphate 100 mM (Promega, WI, USA)
Mineral oil (BRL, USA)
99% Glycerol (BRL, USA)
RNA_{se} inhibitor 40,000 U/ml (Promega, WI, USA)
Reverse transcriptase (AMV) 8,000-10,000 U/ml พร้อม
5x RT buffer (Promega, WI, USA)
Taq DNA polymerase 5,000 U/ml พร้อม 10x Taq DNA
polymerase buffer (Promega, WI, USA)
Primer I (17 bases) 5' CTA CAA TGG ATG CCG AC 3'
(Synthetic genetics, USA)
Primer II (18 bases) 5' GAG TCA CTC GAA TAT GTC 3'
(Synthetic genetics, USA)

Primer III (19 bases) 5' GAC ATG TCC GGA AGA CTG G 3'

(Synthetic genetics, USA)

Primer IV (20 bases) 5' GTA TTG CCT CTC TAG CGG TG 3'

(Synthetic genetics, USA)

DNA/Hind III size markers 500 μ g (BRL, USA)

2. อุปกรณ์เครื่องใช้

2.1 วัสดุพลาสติก

Polypropylene, round-bottom tube with cap size

17x100 mm (Becton Dickinson Co, USA)

Centrifuge tube 50 ml with screwcap (Nunc,

Denmark)

Micro-centrifuge tube 1.5 ml, 0.6 ml (Elkay

Products, Inc., USA)

2.2 เครื่องแก้ว

Serological pipettes (Pyrex, Corning, NY, USA)

Erlenmeyer flask (Pyrex, Corning, NY, USA)

beaker (Pyrex, Corning, NY, USA)

Cylinder (Pyrex, Corning, NY, USA)

3. เครื่องมือ

Homoginizer : Ultra-turrax T 25 (Janke-kunkel,
Germany)

Biohazard

Automatic pipettes (Gilson, France)

Microcentrifuge (Hermile, Germany)

Speed vacuum (UniEquip, Germany)

Spectrophotometer DU-6 (Beckman, USA)

DNA Thermal Cycler 48 wells (Perkin Elmer Cetus, USA)

Horizontal apparatus and gel casting system (BRL,
USA)

Power supply (Pharmacia LKB, Sweden)

Mixer vortex (Scientific industries, USA)

UV transluminator (Fotodyne, Inc., USA)

FRC-10 camera system (Fotodyne, Inc., USA)

ภาคผนวก ข

การเตรียมน้ำยา

1. การเตรียมน้ำยาสำหรับสกัด RNA

- 1.1 0.75 M Sodium citrate pH 7.0
Tri-sodium citrate 22.05 g
ปรับ pH ด้วย glacial acetic acid
เติมน้ำกลั่นให้ครบ 100 ml
ทำให้ปราศจากเชื้อโดย autoclave
- 1.2 Diethylpyrocarbonate water (DEPC·H₂O)
DEPC 1 ml
เติมน้ำกลั่นให้ครบ 1,000 ml
ทำลาย DEPC โดยการ autoclave

1.3 Guanidium thiocyanate stock solution
(GTC stock solution)

| | | |
|------------------------------|------|----|
| guanidine thiocyanate salt | 250 | g |
| 0.75 M sodium citrate pH 7.0 | 17.6 | ml |
| น้ำกลั่น | 293 | ml |

เก็บในขวดสีน้ำตาล

1.4 10% Sarcosyl

| | | |
|----------|-----|----|
| Sarcosyl | 10 | g |
| น้ำกลั่น | 100 | ml |

1.5 Guanidium thiocyanate working solution
(Denaturing solution)

| | | |
|--------------------|------|----|
| GTC stock solution | 47.1 | ml |
| 10% Sarcosyl | 2.5 | ml |
| 2-mercaptoethanol | 0.4 | ml |

เตรียมก่อนใช้

1.6 Phenol (nucleic acid grade)

ละลาย phenol ที่ 65 °C

เติม DEPC·H₂O เท่าตัว

วางไว้จนกระทั่ง phenol แยกจาก DEPC·H₂O ชัดเจน

เก็บในขวดสีน้ำตาล หรือห่อด้วยกระดาษพอยส์

หมายเหตุ saturated phenol สามารถเก็บไว้ได้

นาน 1 เดือนที่ 4 °C

1.7 5 M NaOH

| | | |
|-------------------|-------|----|
| NaOH | 200 | g |
| เติมน้ำกลั่นจนครบ | 1,000 | ml |

1.8 2 M NaOH

| | | |
|-------------------|-------|----|
| 5 M NaOH | 400 | ml |
| เติมน้ำกลั่นจนครบ | 1,000 | ml |

1.9 Chloroform : isoamylalcohol 45:1

| | | |
|----------------|---|----|
| Isoamylalcohol | 1 | ml |
|----------------|---|----|

| | | |
|---------------------|----|----|
| เติม chloroform ครบ | 50 | ml |
|---------------------|----|----|

เก็บในขวดสีน้ำตาล หรือ ห่อด้วยกระดาษพอยส์

1.10 2 M Sodium acetate pH 4.0

| | | |
|----------------|--------|---|
| Sodium acetate | 162.02 | g |
|----------------|--------|---|

ปรับ pH ด้วย glacial acetic acid

| | | |
|-----------------|-------|----|
| เติมน้ำกลั่นครบ | 1,000 | ml |
|-----------------|-------|----|

พาสเจอร์ไรส์จากเชื้อโรคโดย autoclave

2. การเตรียมน้ำยาสำหรับ PCR

2.1 5 mM dNTP (dATP, dCTP, dGTP, dTTP)

เตรียม 25 mM dNTP จาก 100 mM และ dilute ลง

5 เท่า

2.2 1.25 mM dNTP

เตรียม 1.25 mM dNTP จาก 5 mM dNTP โดย dilute ลง 4 เท่า

3. การเตรียมน้ำยาสำหรับ gel electrophoresis

3.1 50x TAE buffer stock solution

| | | |
|---------------------|-------|----|
| Trisma base | 242 | g |
| Glacial acetic acid | 57.1 | ml |
| 0.5 M EDTA pH 8.0 | 100 | ml |
| เติมน้ำกลั่นครบ | 1,000 | ml |

ทำให้ปราศจากเชื้อโดย autoclave

3.2 1x TAE buffer working solution

| | | |
|-----------------|-------|----|
| 50x TAE | 20 | ml |
| เติมน้ำกลั่นครบ | 1,000 | ml |

3.3 0.5 M EDTA pH 8.0

| | | |
|-----------------|------|----|
| EDTA | 18.6 | g |
| เติมน้ำกลั่นครบ | 100 | ml |

ปรับ pH ด้วย NaOH

3.4 1.2% agarose

Agarose 3 g

1x TAE 250 ml

ต้ม agarose ด้วย microwave oven จนกระทั่งใส

เติม ethidium bromide 12.5 ul, mix ให้เข้ากัน

ตั้งทิ้งไว้ให้มีอุณหภูมิประมาณ 55-60 °C ก่อนเท gel

3.5 Ethidium bromide

Ethidium bromide 0.1 g

เติมน้ำกลั่นครบ 10 ml

3.6 Gel loading buffer (Type I)

0.25% bromophenol blue

0.25% Xylene cyanol

40% (W/V) sucrose in distilled water

เก็บไว้ที่ 4 °C

ประวัติผู้เขียน

ราตรี รัตนศิริภักษ์ เกิดเมื่อวันที่ 3 มีนาคม พศ. 2508 ที่จังหวัดบึงกาฬสำเร็จการศึกษาปริญญาวิทยาศาสตรบัณฑิต (เทคนิคการแพทย์) จากมหาวิทยาลัยขอนแก่น เมื่อปี พศ. 2531 เข้ารับราชการที่ รพ.ธรรมศาสตร์ มหาวิทยาลัยธรรมศาสตร์ ในตำแหน่งนักวิทยาศาสตร์ตั้งแต่ปี พศ. 2531 จนถึงปัจจุบัน

