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ส่วนผนวก
วิธีการเตรียมสารเคมี

Acrylamide gel

สารละลาย 50% acrylamide ปริมาตร 100 มิลลิลิตร เตรียมโดยชั่ง acrylamide 49.2 กรัม และ N,N'-methylenebisacrylamide 0.8 กรัม ละลายในน้ำกลั่น คนจนละลายสมบูรณ์ ปรับปริมาตรด้วยน้ำกลั่นจนครบ 100 มิลลิลิตร เก็บในภาชนะป้องกันแสงที่อุณหภูมิห้อง

4x separating buffer (100 มิลลิลิตร)

1.5 M	Tris base 2M	75	มิลลิลิตร
0.4%	SDS 10%	4	มิลลิลิตร
	Ultrapure water	1	มิลลิลิตร
	HCl ปรับจนได้ pH 8.8		
	Ultrapure water เต็มจนครบ	100	มิลลิลิตร

4x stacking buffer (100 มิลลิลิตร)

0.5 M	Tris base 2M	25	มิลลิลิตร
0.4%	SDS 10%	4	มิลลิลิตร
	Ultrapure water	70	มิลลิลิตร
	HCl ปรับจนได้ pH 6.8		
	Ultrapure water เต็มจนครบ	100	มิลลิลิตร

4x sample buffer (20 มิลลิลิตร)

8%	SDS	1.6	กรัม
	2-mercaptoethanol 20%	4	มิลลิลิตร
	125 mM Tris 500 mM, pH 6.8	5	มิลลิลิตร
0.02%	bromophenol blue	4	มิลลิกรัม
40%	glycerol	8	มิลลิลิตร
	Ultrapure water เต็มจนครบ	20	มิลลิลิตร

Ammonium persulfate (APS)

ชั่ง APS กรัม ละลายในน้ำกลั่น คนจนละลาย เต็มน้ำจนครบ 100 มิลลิลิตร

Separating gel

เตรียม acrylamide gel จำนวน 2 แผ่น ส่วนผสมแต่ละแผ่น มีดังนี้

	8%	12%	
Ultrapure water	5.9	5.1	มิลลิลิตร
4x separating buffer	2.5	2.5	มิลลิลิตร
50% acrylamide	1.6	2.4	มิลลิลิตร
10% APS	50	50	ไมโครลิตร
TEMED	10	10	ไมโครลิตร

ผสมส่วนผสมทั้งหมดให้เข้ากันอย่างสมบูรณ์ เทในช่องระหว่างแผ่นกระจกทันที หลังจากนั้นเทน้ำกลั่นให้มีความสูงประมาณ 4-5 มิลลิเมตร เพื่อปรับหน้าเจลให้เรียบ ตั้งทิ้งไว้ 20-30 นาที เพื่อให้เจลแข็งตัว

Stacking gel

หลังจาก separating gel แข็งตัวอย่างสมบูรณ์แล้ว เทน้ำกลั่นที่ใช้ปรับหน้าเจลออก และทำการผสมส่วนผสม ดังนี้

Ultrapure water	2.6	มิลลิลิตร
4x stacking buffer	1.0	มิลลิลิตร
50% acrylamide	0.4	มิลลิลิตร
10% APS	30	ไมโครลิตร
TEMED	5	ไมโครลิตร

ผสมส่วนผสมทั้งหมดให้เข้ากันอย่างสมบูรณ์ เทในช่องระหว่างแผ่นกระจกด้านบนทันที หลังจากนั้นเสียบ comb ลงไปในเจล ตั้งทิ้งไว้ 20-30 นาที เพื่อให้เจลแข็งตัว

การหยอดตัวอย่าง

หลังจากที่ stacking gel แข็งตัวอย่างสมบูรณ์ ทำการดึง comb ออกอย่างระมัดระวัง ใช้ electrophoresis buffer ทำความสะอาดหลุม หยอดตัวอย่างลงในหลุม

Bradford reagent

เตรียม Bradford reagent ปริมาตร 1 ลิตร โดยมีส่วนประกอบดังนี้ Coomassie Brilliant Blue G-250 50 มิลลิกรัม เมทานอล 25 มิลลิลิตร และ 85% phosphoric acid 50 มิลลิลิตร ผสมให้เข้ากันและปรับปริมาตรด้วยน้ำกลั่นจนครบ 500 มิลลิลิตร นำไปกรองผ่านกระดาษกรอง Whatman เบอร์ 93 เก็บในภาชนะปิดสนิทและป้องกันแสงที่อุณหภูมิ 4 องศา

Lysis buffer สำหรับการทดลอง Western blot

เตรียม 2x lysis buffer ปริมาตร 30 มิลลิลิตร ประกอบด้วย Tris-HCl (pH 7.4) 40 มิลลิโมลาร์ NaCl 300 มิลลิโมลาร์ Triton-X 2% sodium deoxycholate 2% NaF 20 มิลลิโมลาร์ของ Pefabloc 2 มิลลิโมลาร์ และ sodium orthovanadate 2 มิลลิโมลาร์ โดยละลายส่วนผสมทั้งหมดในน้ำกลั่น และปรับปริมาตรจนครบ 30 มิลลิลิตร ก่อนนำไปใช้ เติม protease inhibitor ปริมาตร 10 ไมโครลิตรต่อ lysis buffer 990 ไมโครลิตร

M199 medium

อาหารเพาะเลี้ยง M199 แบบผง (1 ซอง) ละลายในน้ำกลั่น เติม sodium hydrogen carbonate 2.2 กรัม ผสมให้เข้ากัน ปรับ pH ให้ได้ 7.2 ด้วยกรด HCl ปรับปริมาตรด้วยน้ำกลั่นจนครบ 1 ลิตร ทำให้ปราศจากเชื้อโดยกรองผ่านเมมเบรนขนาด 0.22 ไมโครเมตร เติม FBS และ penicillin-streptomycin จนได้ความเข้มข้นสุดท้ายเป็น 10% FBS และ 1% penicillin-streptomycin

Phosphate buffer saline (PBS)

เตรียม PBS ปริมาตร 1 ลิตร ประกอบด้วย NaCl 8 กรัม KCl 0.2 กรัม KH_2PO_4 0.2 กรัม และ Na_2HPO_4 1.15 กรัม โดยละลายในน้ำกลั่น ปรับ pH จนได้ 7.4 ด้วย NaOH หลังจากนั้นปรับปริมาตรด้วยน้ำกลั่นจนครบ 1 ลิตร

Running and transfer solution สำหรับการทดลอง Western blot

10x running and transfer solution

เตรียม 10x running and transfer solution ปริมาตร 1 ลิตร ประกอบด้วย Tris (pH 8.3) 250 มิลลิโมลาร์ และ glycine 1.92 โมลาร์ โดยละลายส่วนผสมในน้ำกลั่น และปรับปริมาตรจนครบ 1 ลิตร

1x running buffer

เตรียม 1x running buffer ปริมาตร 1 ลิตร ทำโดยผสม 10x running and transfer solution 100 มิลลิลิตร ผสมกับ 10% SDS 10 มิลลิลิตร ปรับปริมาตรด้วยน้ำกลั่นจนครบ 1 ลิตร

1x transfer buffer

เตรียม 1x transfer buffer ปริมาตร 1 ลิตร ทำโดยผสม 10x running and transfer solution 80 มิลลิลิตร ผสมกับ methanol 220 มิลลิลิตร ปรับปริมาตรด้วยน้ำกลั่นจนครบ 1 ลิตร

**5x sample buffer สำหรับการทดลอง Western blot**

เตรียม 5x sample buffer ปริมาตร 50 มิลลิลิตร ประกอบด้วย Tris-HCl (pH 6.8) 60 มิลลิโมลาร์ SDS 2% glycerol 25% β -mercaptoethanol 14.4 มิลลิโมลาร์ และ bromophenol blue 0.1% โดยละลายในน้ำกลั่นและปรับปริมาตรด้วยจนครบ 50 มิลลิลิตร เก็บไว้ที่อุณหภูมิ -20 องศา

10x Tris-buffered saline (TBS) สำหรับการทดลอง Western blot

เตรียม 10x TBS ปริมาตร 1 ลิตร ประกอบด้วย Tris (2M, pH 7.5) 50 มิลลิลิตร และ NaCl 87.6 กรัม ละลายน้ำกลั่นและปรับปริมาตรจนครบ 1 ลิตร

1x Tris-buffered saline, 0.05% Tween 20 (TBST)

เตรียม 1x TBST ปริมาตร 1 ลิตร เตรียมโดยผสม 10x TBS 100 มิลลิลิตร กับ Tween 20 ปริมาตร 0.5 มิลลิลิตร ปรับปริมาตรด้วยน้ำกลั่นจนครบ 1 ลิตร

