BUKU PEMBELAJARAN		
Mata Kuliah	:	**BIOLOGI SEL DAN JARINGAN**
Kode Mata Kuliah	:	**BS-1**
Prodi/Jurusan	:	**PENDIDIKAN DOKTER**
Fakultas	:	**KEDOKTERAN**
Judul Buku	:	**PETUNJUK PRAKTIKUM ELEKTROFORESIS**

Disusun oleh:

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Universitas Trisakti

Jakarta

2021

TUJUAN PRAKTIKUM

1. Untuk mengetahui bagian alat elektroforesis

2. Untuk mengetahui reagen yang digunakan

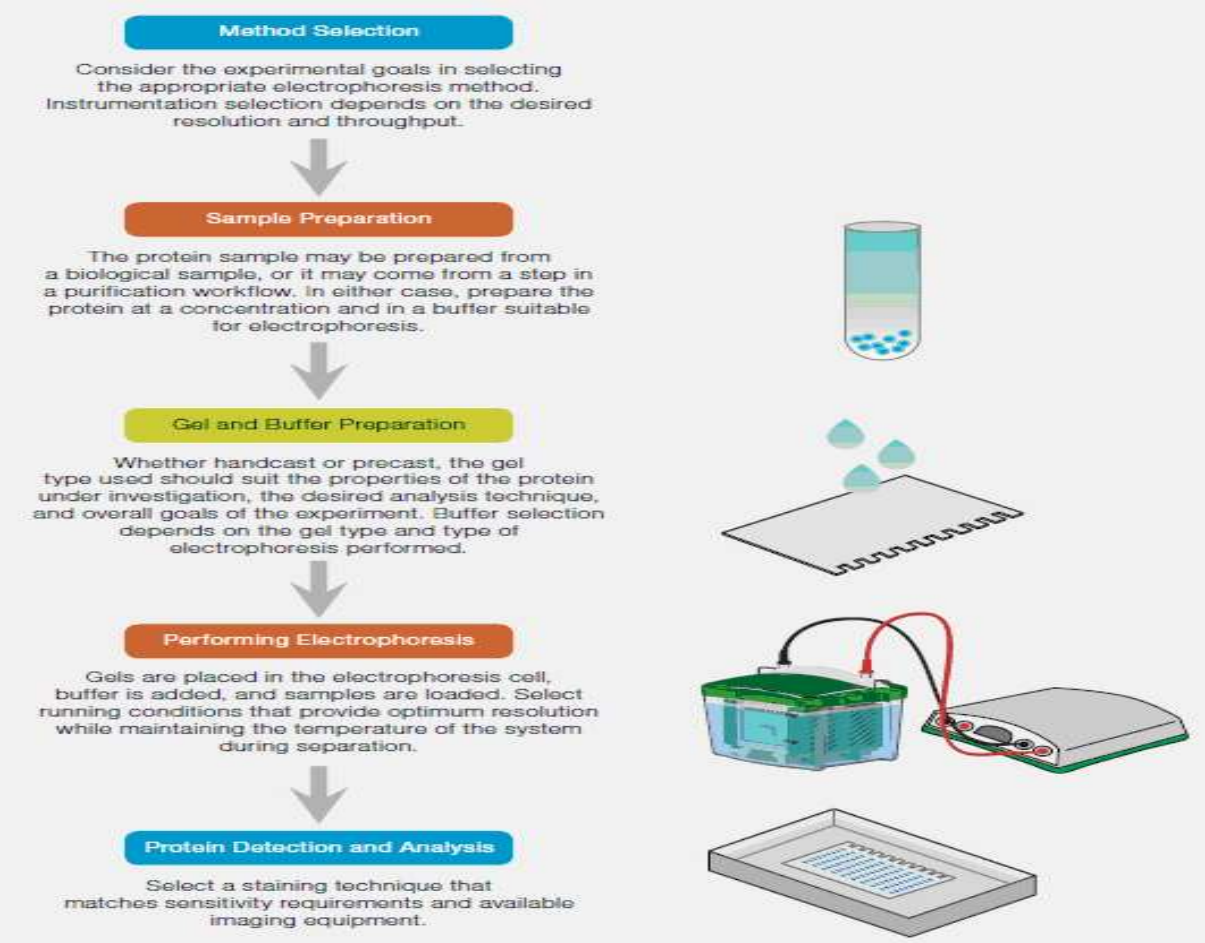
3. dalam elektroforesis diharapkan dapat:

    a. Dapat mengoperasikan alat elektroforesis

    b. Dapat membaca hasil elektroforesis

SKEMA ELEKTROFORESIS

SKEMA ELEKTROFORESIS

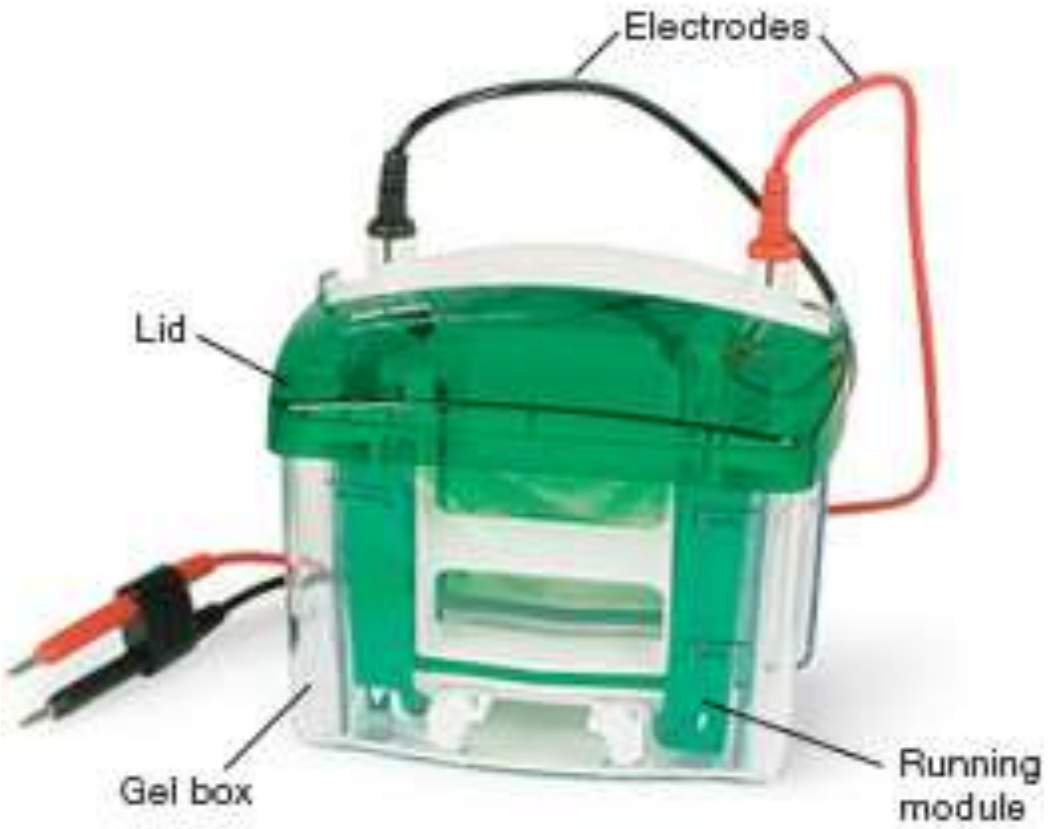


1. ALAT ELEKTROFORESIS

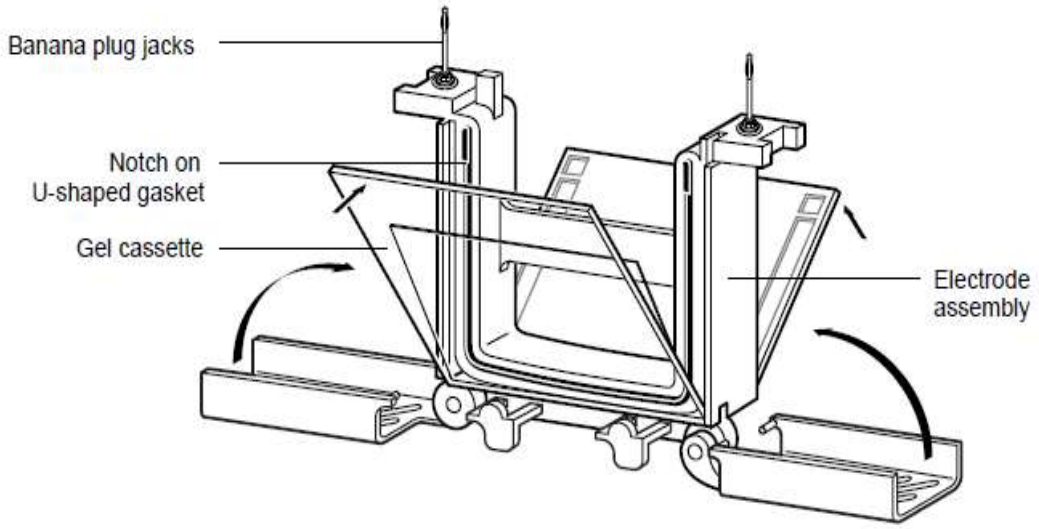
- Alat minigel (*Bio-Rad Mini Protean electrophoresis apparatus*).
- Power supply (kapasitas 200 V, 500 mA).
- *Waterbath*.
- Sentrifug Eppendorf (*Eppendorf centrifuge*).
- *Hamilton syringes* (kapasitas 25 µL).
- Pengering gel.
- Pompa pengisap.
- Wadah palstik.
- Tabung Eppendorf (*Eppendorf tubes*).
- *Shaker*.
- Mikropipet

- Alat minigel (*Bio-Rad Mini Protean electrophoresis apparatus*).

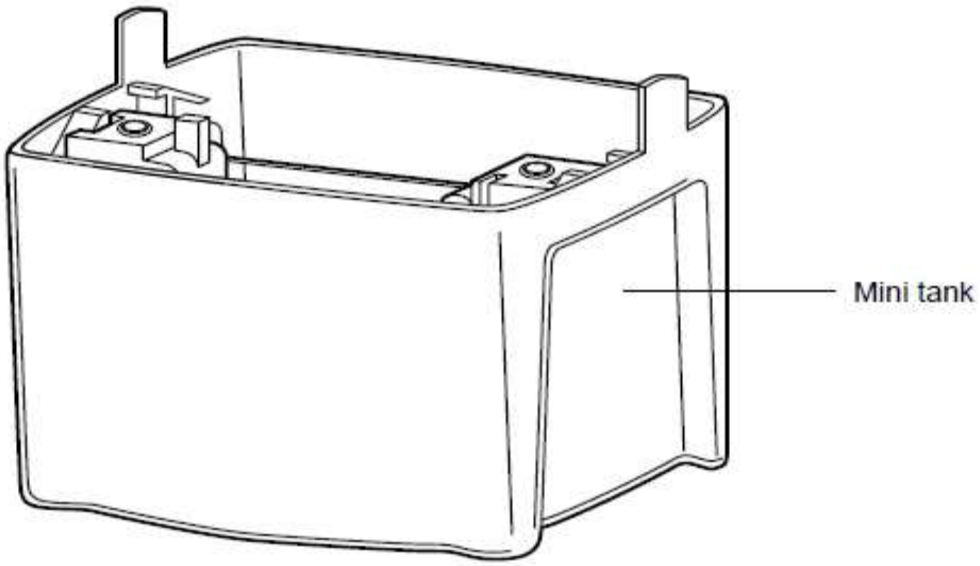
SUSUNAN ALAT ELECTROFORESIS



RUNNING MODULE

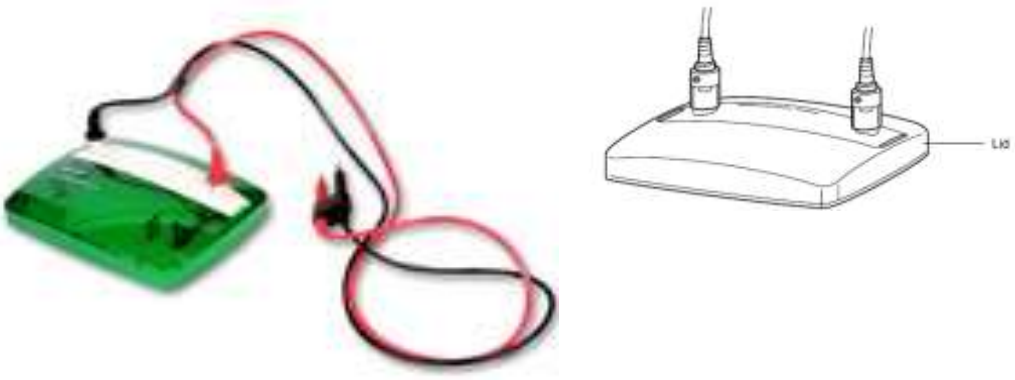


MINI TANK (BUFFER TANK)



Buffer Tank and Lid

Cell Lid With Power Cables



Power Cables

POWER SUPLY

kapasitas 200 V, 500 mA

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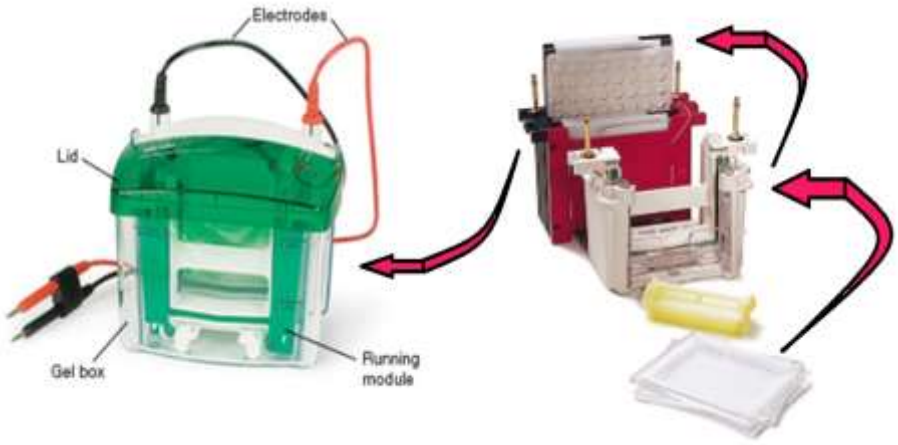

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Water bath.

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Sentrifuge

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Hamilton syringes

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Tabung Eppendorf

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SHAKER

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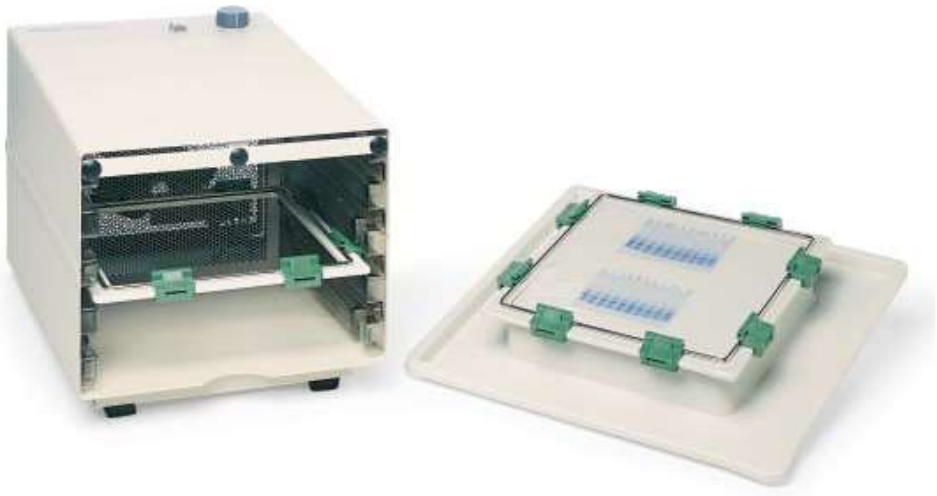
PENGERING GEL



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MIKRO PIPET

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2. REAGEN ELEKTROFORESIS

- REDUCING SAMPLE BUFFER (RSB)
- SEPARATING GEL (RUNNING GEL= GEL SDS PAGE)
- STACKING GEL
- RUNNING BUFFER
- Stain:

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KOMPOSISI RSB

- RSB=Electrophoresis Sample Buffer → 2X is a ready-to-use reducing electrophoresis sample buffer solution with bromophenol blue for the preparation of protein samples to be separated in SDS-PAGE.
- SDS 10%
- DTT 500 mM
- Glycerol 50%
- Tris-HCL 250 mM
- Bromophenol blue dye 0.5%
- pH →6.8

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KOMPOSISI GEL SDS PAGE 10%

Running/Main Gel		12.5%		10%	15%
No	Materials	1 slap (µL)	2 slap (µL)	1 slap (µL)	1 slap (µL)
1.	Acrylamide 30%	2063	4126	1650	2470
2.	TrisHCl 1.5 M pH 8.8	1250	2500	1250	1250
3.	Sterile aquades	1635	3270	2050	1230
4.	SDS 10%	50	100	50	50
5.	APS 10%	50	100	12.5	12.5
6.	Temed	10	20	10	10

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KOMPOSISI STACKING GEL

Stacking Gel 3%		12.5%		10%	15%
No	Materials	1 slap (µL)	2 slap (µL)	1 slap (µL)	1 slap (µL)
1.	Acrylamide 30%	257.5	515	412	620
2.	TrisHCl 1 M pH 6.8	312.5	625	625	625
3.	Sterile aquades	662.5	1325	1425	1220
4.	SDS 10%	12.5	25	25	25
5.	APS 10%	3.75	7.5	7.5	7.5
6.	Temed	2.5	5	5	5

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KOMPOSISI RUNNING BUFFER

- **SDS-PAGE 10× SDS Running Buffer**
  - Tris base 30.3 g
  - Glycine144.4 g
  - SDS 10 g
  - Dissolve in 1 L of MilliQ-filtered H_2O.

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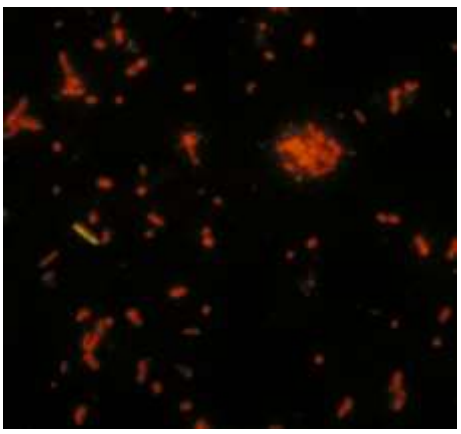
STAIN

- Acridine orange, C.I. 46005, very high purity



Detection of nucleic acids separated by gel electrophoresis.

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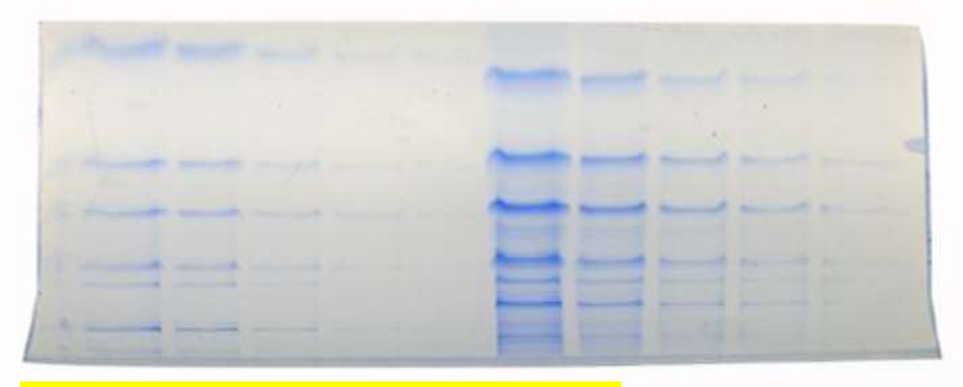
Coomassie® Blue G250, C.I. 42655
Poly G Blue,
Poly R Blue,



protein quantification.

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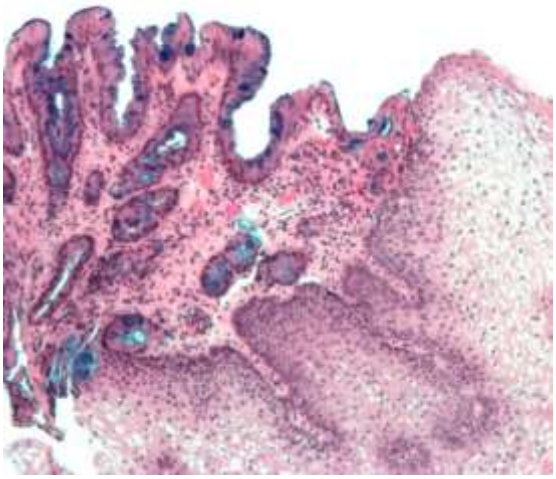
Alcian Blue 8GX, C.I. 74240



Glikoprotein

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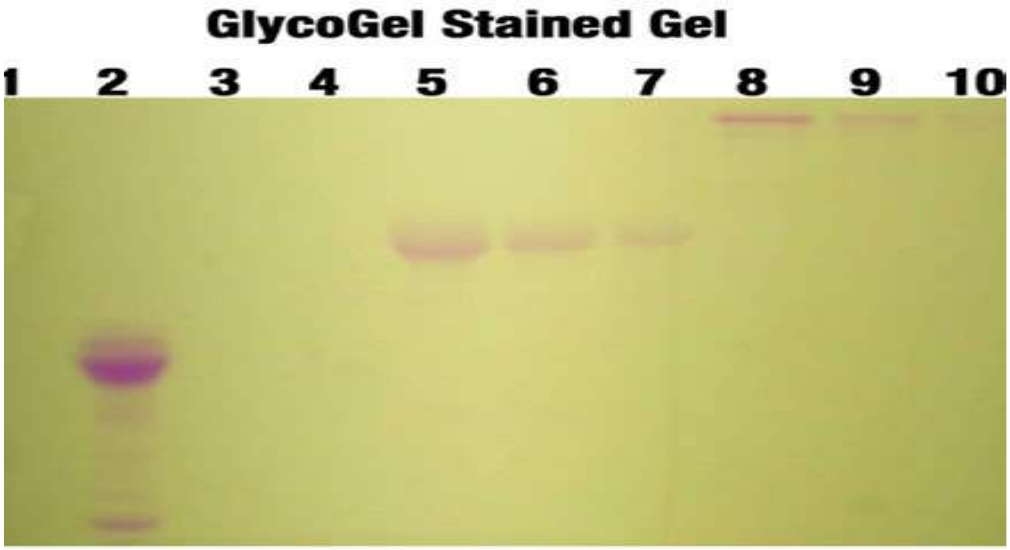
GlycoGel Stain Kit



Glycoprotein detection in gel electrophoresis

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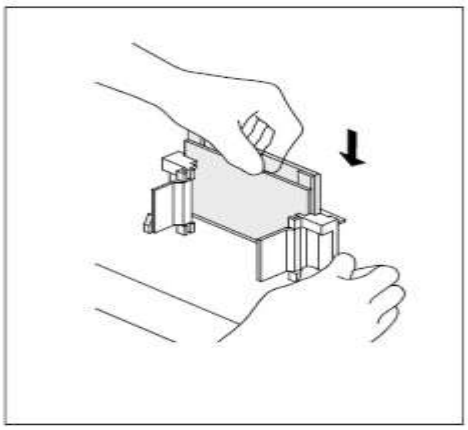
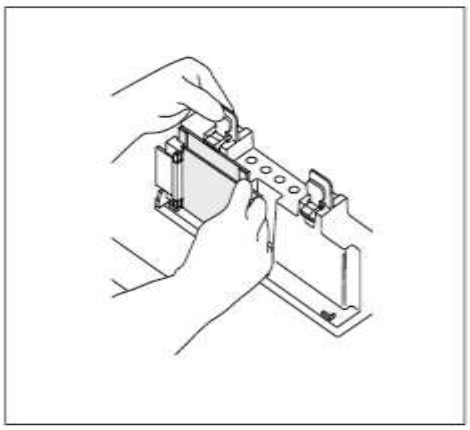
3. CARA KERJA

- Tetra cell casting frame and casting stand





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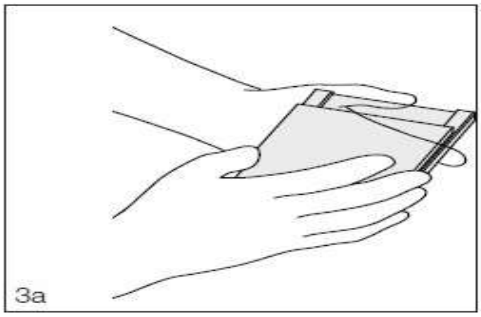
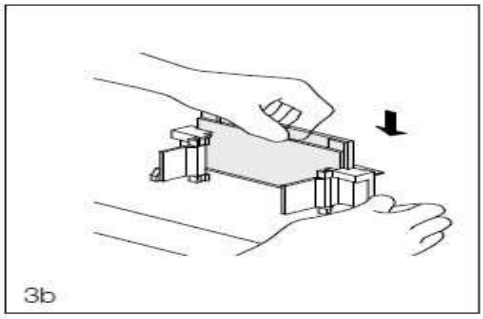
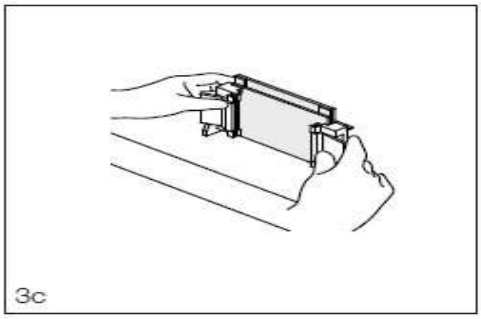
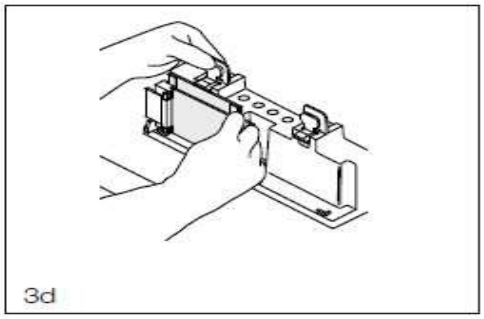
- Tetra cell casting frame and casting stand









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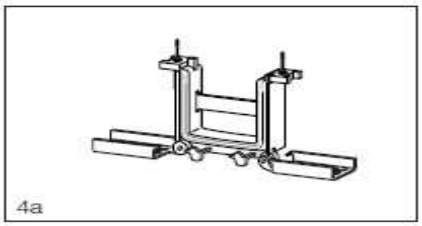
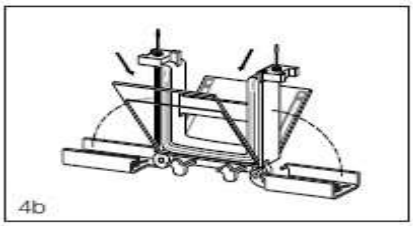
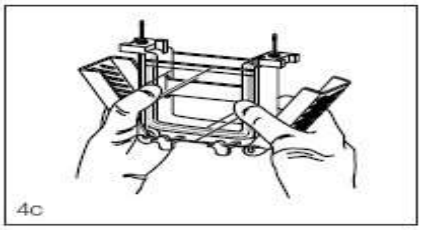
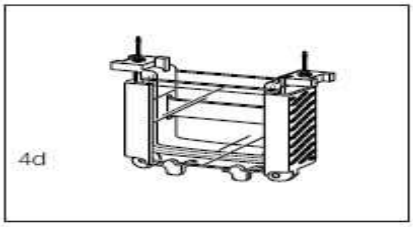
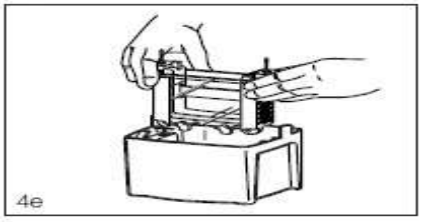
3.1. MEMASANG RUNNING MODULE











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3.2. MENYIAPKAN SAMPLE

1. Sampel protein ditambah dengan Reducing Sample Buffer (RSB)1:1 dalam Tabung Eppendorf.
2. Kemudian sampel dipanaskan pada 100 ^{o}C selama 5 menit
3. Setelah dingin, bila sampel tidak langsung digunakan, sampel bisa simpan pada -20^{o}C

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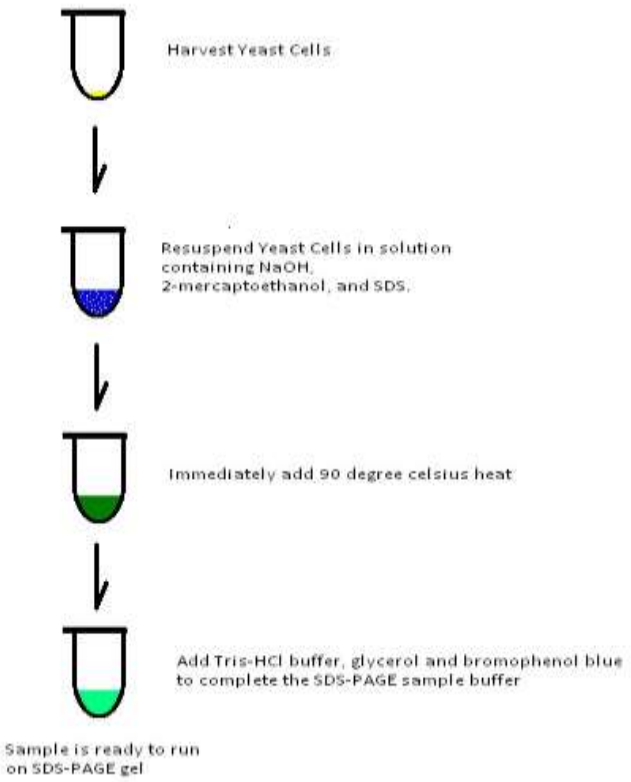

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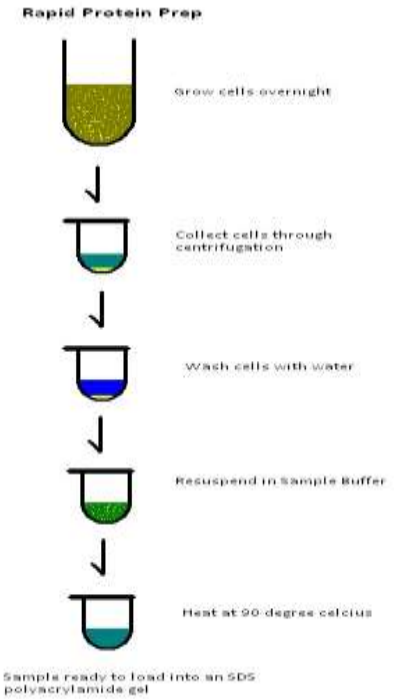
Rapid Protein Prep
Grow cells overnight
Collect cells through centrifugation
Wash cells with water
Resuspend in Sample Buffer
Heat at 90 degree celcius
Sample ready to load into an SDS polyacrylamide gel

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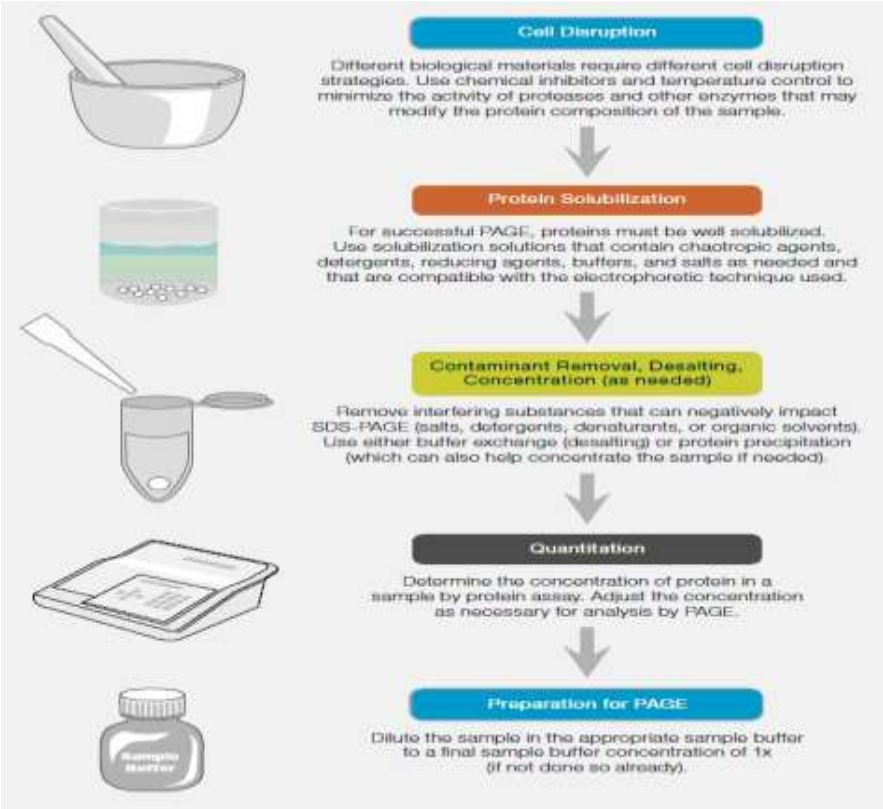
Cell Disruption
Different biological materials require different cell disruption strategies. Use chemical inhibitors and temperature control to minimize the activity of proteases and other enzymes that may modify the protein composition of the sample.
Protein Solubilization
For successful PAGE, proteins must be well solubilized. Use solubilization solutions that contain chaotropic agents, detergents, reducing agents, buffers, and salts as needed and that are compatible with the electrophoretic technique used.
Contaminant Removal, Desalting, Concentration (as needed)
Remove interfering substances that can negatively impact SDS-PAGE (salts, detergents, denaturants, or organic solvents). Use either buffer exchange (desalting) or protein precipitation (which can also help concentrate the sample if needed).
Quantitation
Determine the concentration of protein in a sample by protein assay. Adjust the concentration as necessary for analysis by PAGE.
Preparation for PAGE
Dilute the sample in the appropriate sample buffer to a final sample buffer concentration of 1x (if not done so already).

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3.3. MENYIAPKAN SEPARATING GEL 12.5%

- Siapkan tabung polipropilen 50ml, masukkan aquabidest 1.505 mL, setalah tabung ditutup, goyang perlahan lahan.
- Masukkan SDS 10% 75µl, setelah tabung ditutup, goyang perlahan-lahan.
- Masukkan APS 10% 75 µl, setelah tabung ditutup, goyang perlahan-lahan.

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- Masukkan TEMED 6,25 µl, setelah tabung ditutup, goyang perlahan-lahan.
- Menggunakan mikropippet 1 ml, masukkan larutan ke dalam plate pembentuk gel (dijaga jangan sampai terbentuk gelembung udara) sampai batas yang terdapat pada plate.
- Perlahan tambahkan aquadest diatas larutan gel dalam plate agar permukaan gel tidak bergelombang

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- Biarkan gel memadat selama kurang lebih 30 menit (ditandai dengan terbentuknya garis transparan diantara batas air dan gel yang terbentuk).
- Setelah itu, air yang menutup separating gel dibuang.

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3.4. MENYIAPKAN STACKING GEL 3%

Aquabidest	2.11 mL
Acrylamid-bis 30%	0.45 mL
Tris 1 M, pH 6.8	0.38 mL
SDS 10%	30 uL
APS 10%	30 uL
TEMED	5 uL

Tahapan pembuatan stcaking gel sama dengan separating gel

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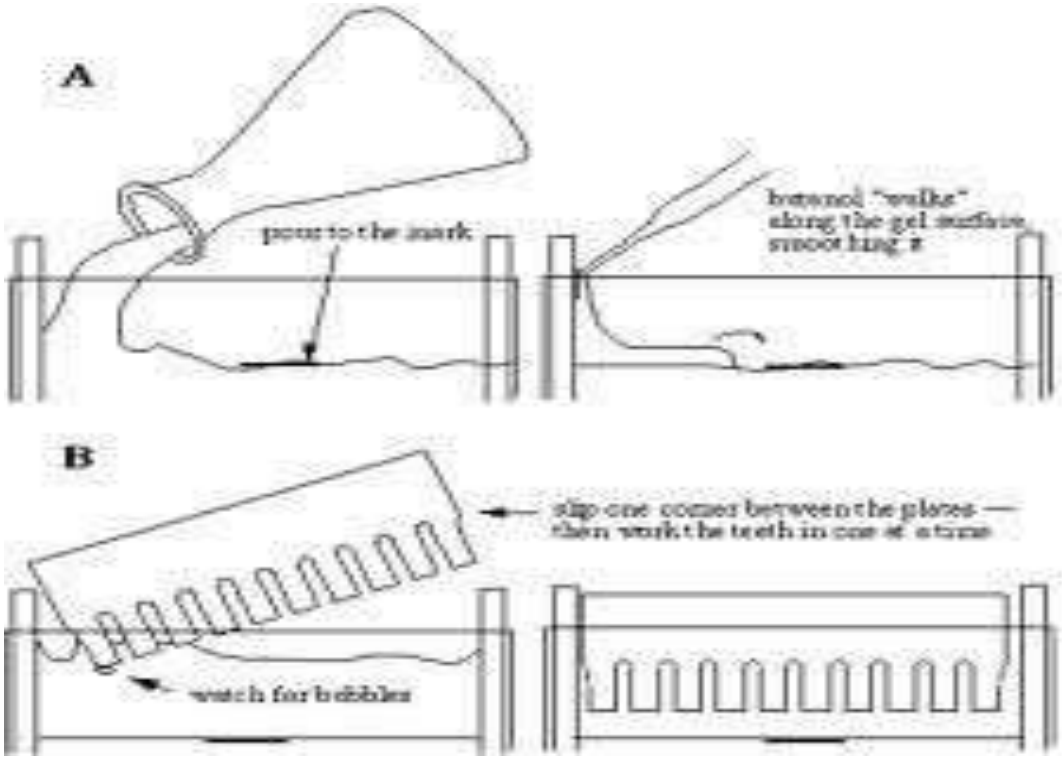

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10-Well Comb

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Ready Gels Accelerate Your Research!

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LOADING

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1. Plate yang sudah berisi gel di masukkan dalam chamber elektroforesis
2. Running buffer dituang sampai bagian atas sehingga gel terendam
3. Apabila terbentuk gelembung udara pada dasar gel atau diantara sumur sampel, udara harus dihilangkan
4. Marker standar 3 - 5 uL dimasukkan pada salah satu sumur (bisa disumur yang paling tepi atau pada sumur yang tengah)

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5. Menggunakan Hamiltonsyringe, sampel 10 - 20 µL (kandungan proteinnya minimal 0,1 µg dan maksimal 20 - 40 µg dimasukkan ke dalam dasar sumur gel.

6. Syringe dibilas sampai 3x dengan menggunakan air atau dengan running buffer sebelum dipakai untuk memasukkan sampel yang berbeda pada sumur gel berikutnya.

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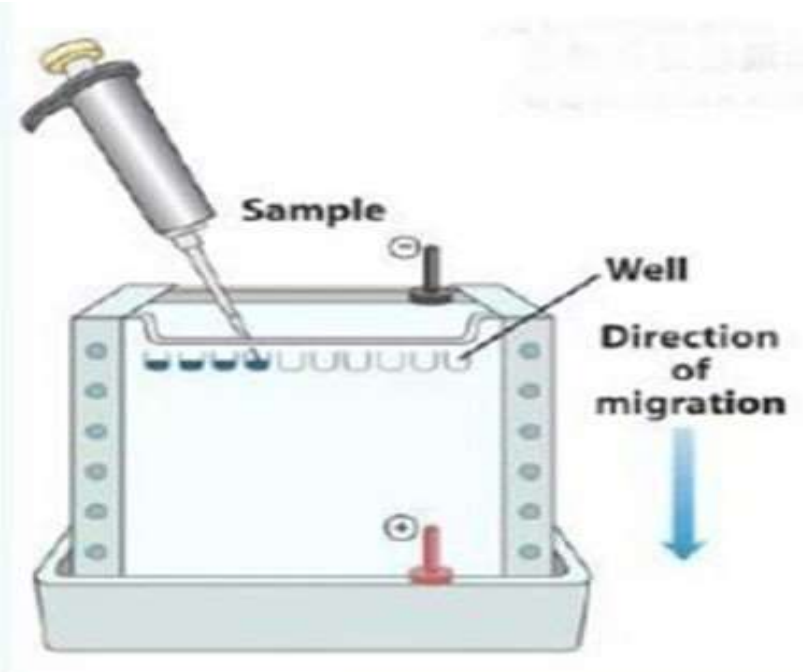
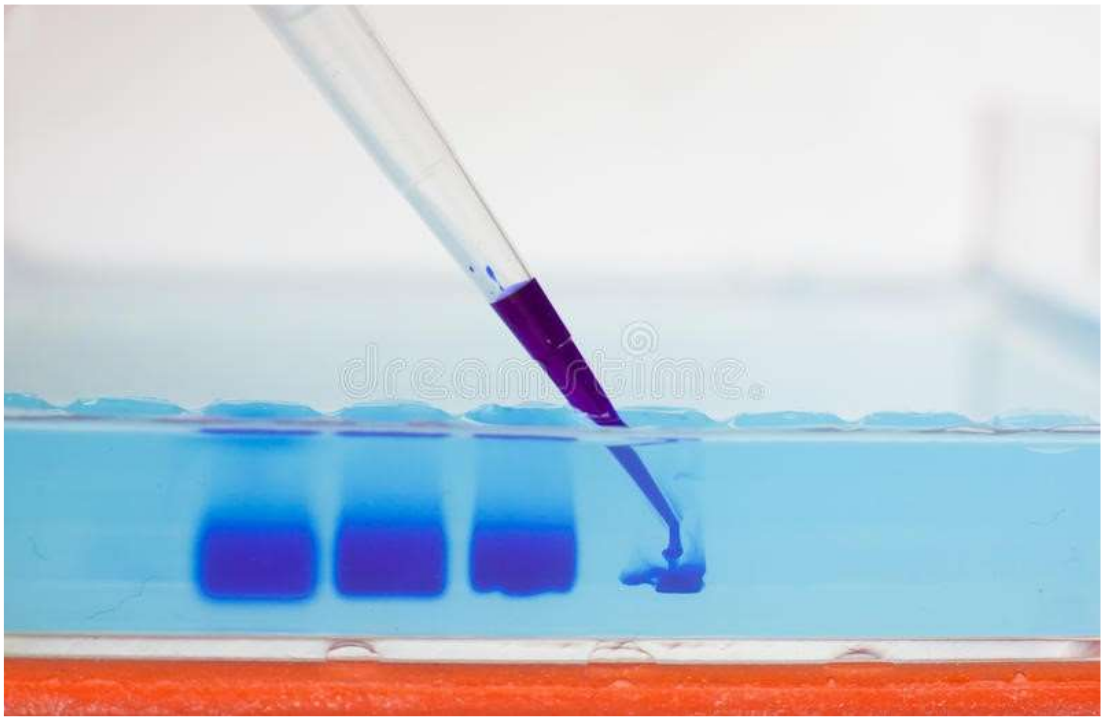
Loading = memasukkan sample kedalam sumur (well)



51



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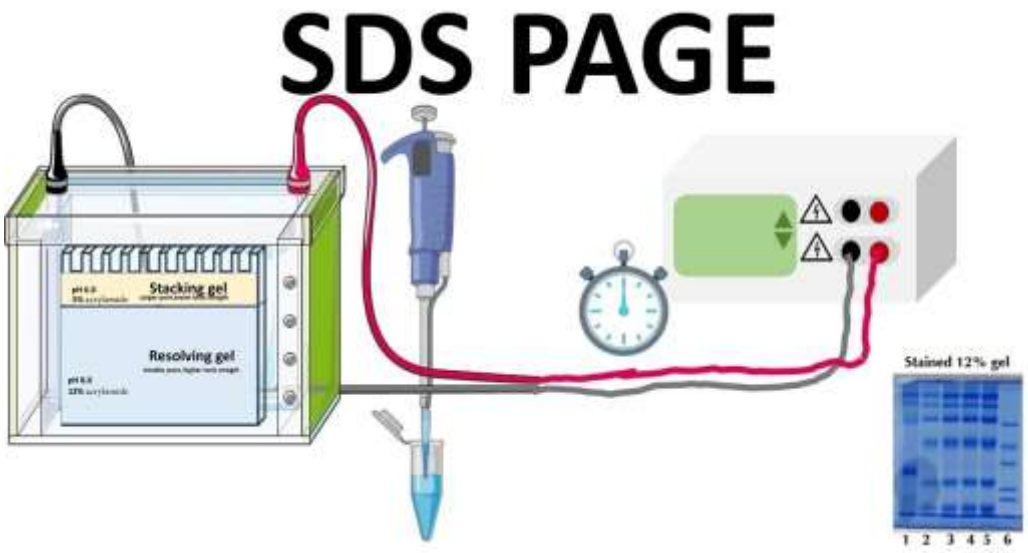
RUNNING



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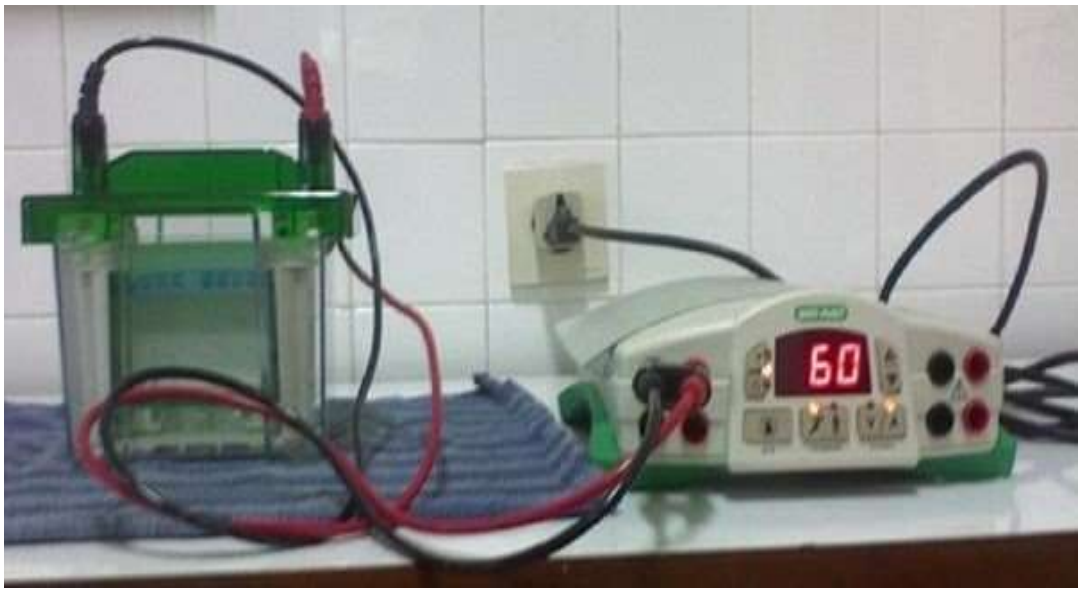

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- Untuk memulai running perangkat elektroforesis dihubungkan dengan power supply
- Running dilakuakn pada constant current 20 mA selama kurang lebih 40-50 menit atau sampai tracking dye mencapai jarak 0,5 cm dari dasar gel
- 3. Setelah selesai, running buffer dituang dan gel diambil dariplate

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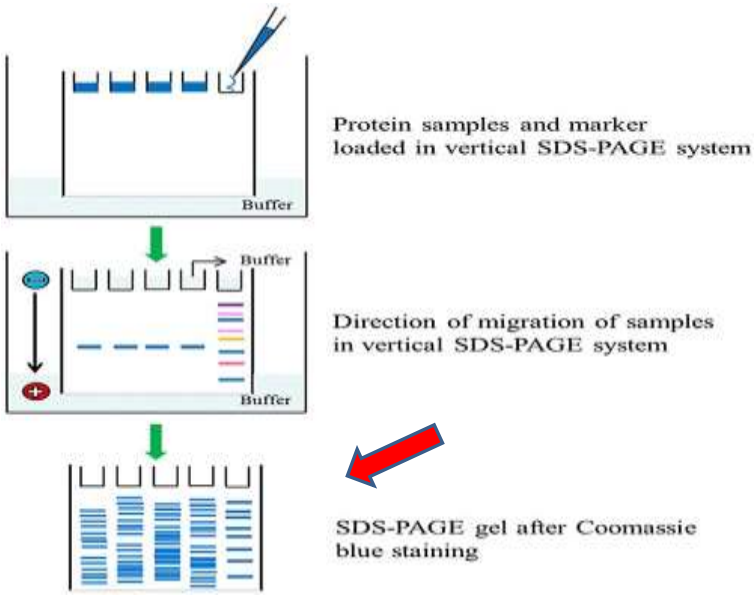
STAINING



Protein samples and marker loaded in vertical SDS-PAGE system

Direction of migration of samples in vertical SDS-PAGE system

SDS-PAGE gel after Coomassie blue staining

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- Untuk tahap ini diperlukan larutan staining untuk mewarnai protein gel
- Pewarnaan yang dipakai adalah Comasie Brilliant Blue atau Silver Stain atau yang lain, tergantung kegunaan.
- Staining dilakukan selama 30menit
- Larutan destaining untuk menghilangkan warna pada gel dan memperjelas band protein yang terbentuk.

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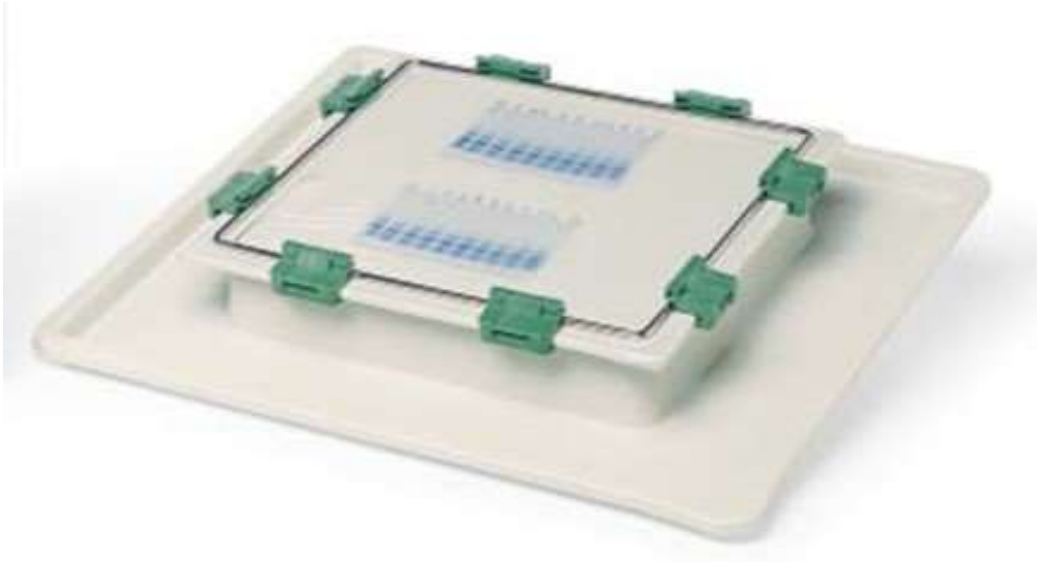
HASIL ELEKTROFORESIS



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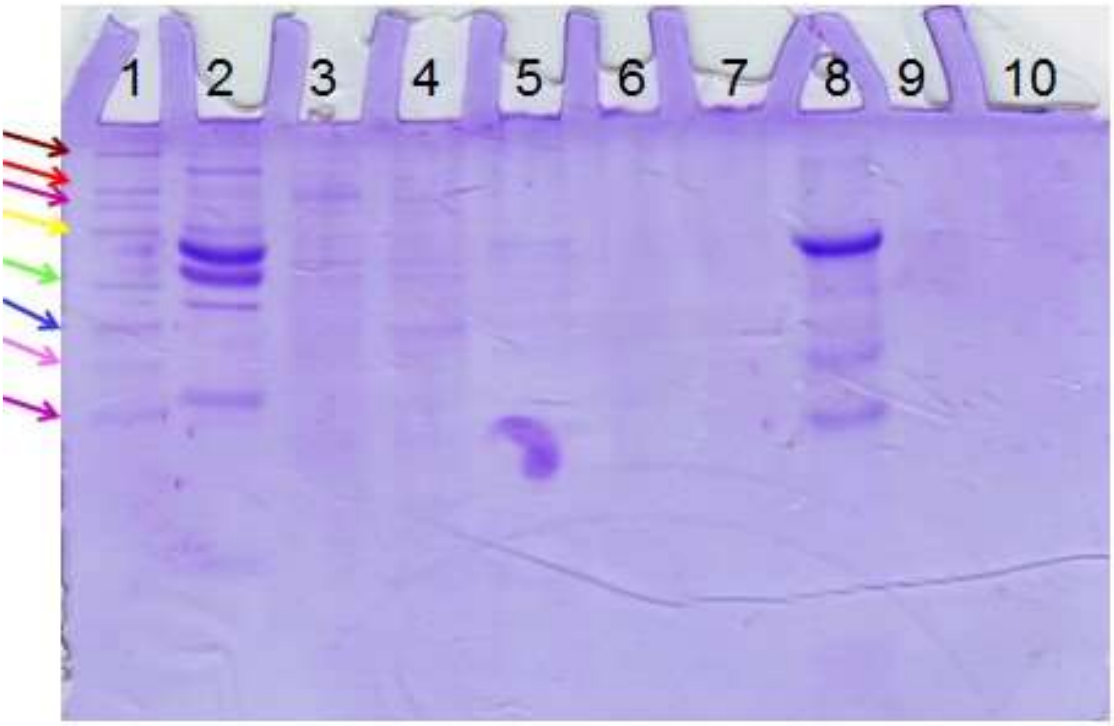

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MOLECULAR WEIGHT

MW Range Resolved	Tris-HCl Gel %T
100-250 kDa	5%
40-200 kDa	7.5%
30-150 kDa	10%
20-120 kDa	12%
10-100 kDa	15%
6-50 kDa	18%
20-250 kDa	4-15%
10-200 kDa	4-20%
6-70 kDa	8-16%
10-100 kDa	10-20%

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CARA MEMBACA HASIL EF

- Protein marker (well 1)

Band	MW Marker	Tracking distance	Log MW	RF
1	260	2	2.414973348	0.029412
2	135	6.5	2.130333768	0.095588
3	95	8	1.977723605	0.117647
4	72	10.5	1.857332496	0.154412
5	52	17	1.716003344	0.25
6	42	21.5	1.62324929	0.316176
7	34	26	1.531478917	0.382353
8	26	31.5	1.414973348	0.463235

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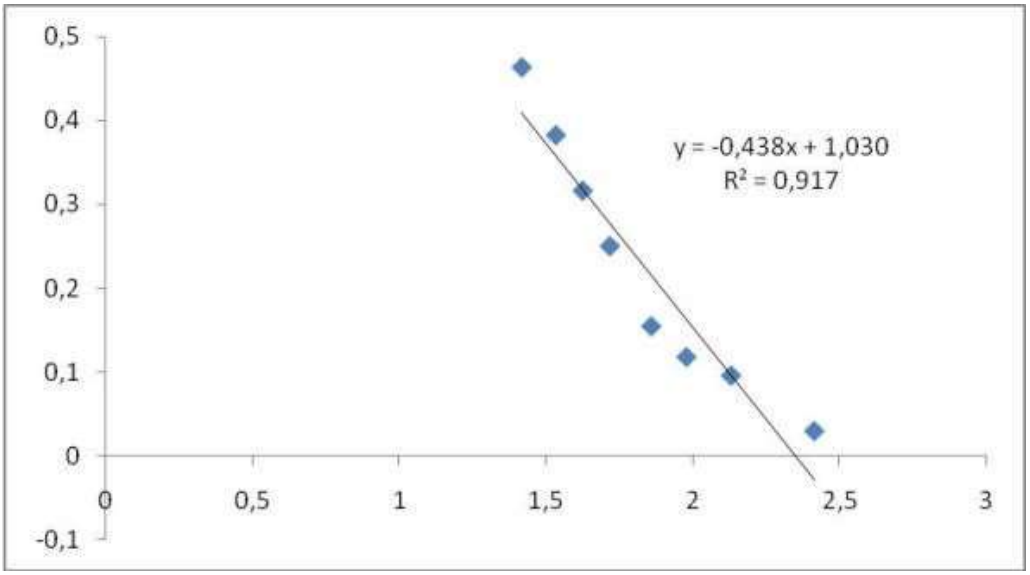
GRAFIK BM



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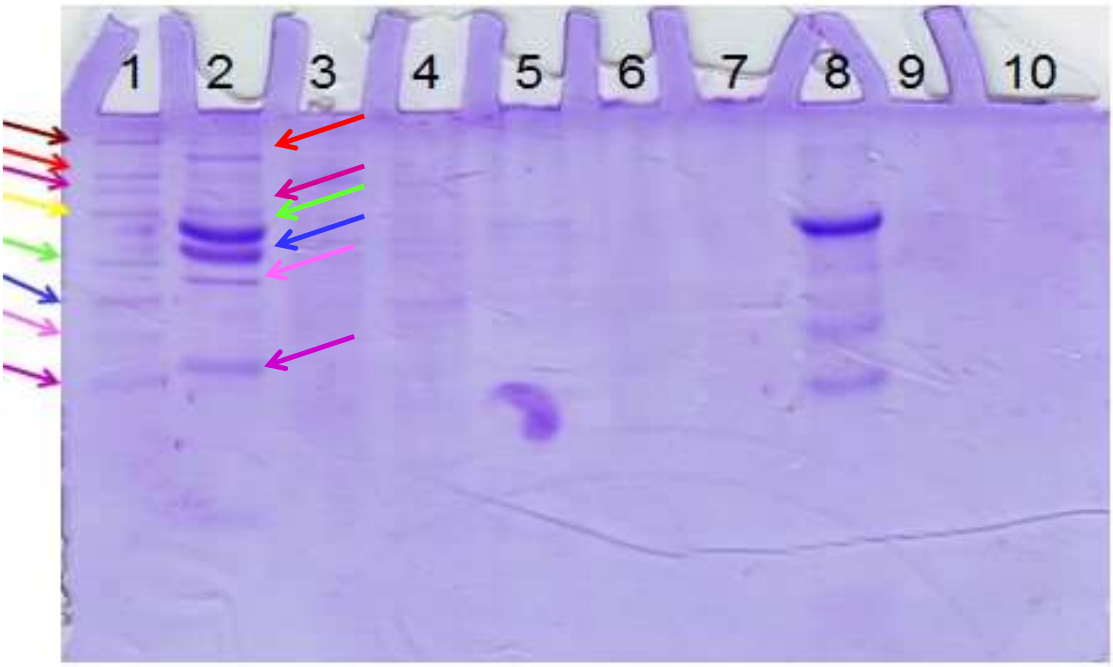
WELL 2



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BM PROTEIN WELL 2

Band	MW Protein	Tracking distance	Log MW	RF
1	164.9293237	4	2.217297878	0.058824
2	99.78376762	10.5	1.999059898	0.154412
3	82.24740669	13	1.915122213	0.191176
4	67.79294938	15.5	1.831184529	0.227941
5	51.72157842	19	1.71367177	0.279412
6	22.09764323	30	1.344345958	0.441176

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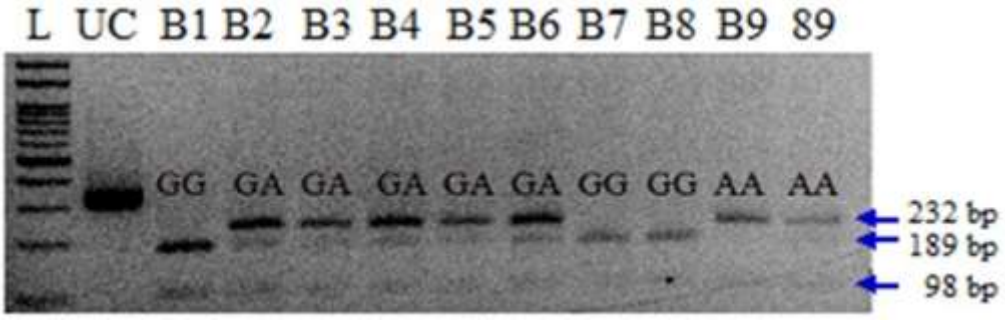
ELEKTROFORESIS DNA



Genotyping of the Fas-promoter-670 gene in the CEC with BstN1 enzyme.
Abbreviations: CEC=cervix-epithelial-cells; L=Molecular weight standard; UC=DNA loading without BstN1 enzyme; 89= sample number of the CEC from thin prep solution showed that genotype of the Fas-promoter-670 gene is GA (232, 189 and 98 bp); C1-C9 =sample control of the CEC from thin prep solution, loading with BstN1;
AA genotype (232, 98 bp)
GA gentoype (232, 189, 98 bp)
GG genotype (189, 98 bp).

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TUGAS UNTUK SETIAP MAHASISWA

Buatlah laporan

Format laporan

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Format laporan praktikum

Nama Mahasiswa :
NIM :

LAPORAN PRAKTIKUM: ELEKTROFORESIS

I. Prinsip dasar:

Xxxxxxxxxx dst...

II. Tujuan praktikum:

1. Xxxxxxxx.....
2. Xxxxxxxx..... dst

III. Alat praktikum

Nomor	Nama alat dan gambar	Fungsi
1.	Xxxxx 	Xxxxxx
2.		

IV. Bahan/Reagen

Nomor	Nama bahan/reagen	Fungsi
1.	Xxxxx	Xxxxxx
2.		

V. Cara kerja elektroforesis

1. Persiapan alat elektroforesis

Xxxxxxxxxxxxxxxxxxxxxxx dst

2. Persiapan sampel

Xxxxxxxxxxxxxxxxxxxxxx dst

3. Membuat running bufer

Xxxxxxxxxxxxxxxxxx dst

4. Membuat separating gel

Xxxxxxxxxxxxxxxxxx dst

5. Membuat stacking gel

Xxxxxxxxxxxxxxxx dst

6. Running

Xxxxxxxxxxxx dst

7. Membaca hasil

Xxxxxxxxxxxx dst

VI. Kesimpulan

Xxxxxxxxxxxx dst.....

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Referensi:

- https://www.youtube.com/watch?v=bdBXwuuwSBo
- https://www.youtube.com/watch?v=XUjLO-ek2C8
- https://www.youtube.com/watch?v=XnEdmk1Sqvg
- https://www.youtube.com/watch?v=K8VFwhYLLm0
- https://www.youtube.com/watch?v=GswMlk2FN3g