Gel Electrophoresis

Recommended Gel Percentages for Separation of Linear DNA

Agarose Gel, %	Range of Separation, bp	Polyacrylamide Gel, %	Range of Separation, bp
0.5	1,000 - 30,000	3.5	100 - 1,000
0.7	800 - 12,000	5	80 - 500
1	500 - 10,000	8	60 - 400
1.2	400 - 7,000	12	40 - 200
1.4	200 - 4,000	20	5 - 100
2	50 - 2,000		

DNA Size Migration with Sample Loading Dyes

Agarose Concentration, %	Xylene cyanol FF	Bromophenol blue	Orange G
0.7 - 1.7	~4000 bp	~300 bp	~50 bp
2.5 - 3.0	~800 bp	~100 bp	~30 bp

Recommended Gel Percentages for Separation of Protein

Recommended % Polyacrylamide	Optimum Resolution for Protein
8	40 – 200 kDa
10	21 – 100 kDa
12	10 – 40 kDa

Migration Rates of the Marker Dyes through Polyacrylamide Gels

Non-denaturing Gels		
Polyacrylamide Gel, %	Xylene cyanol FF	Bromophenol blue
3.5	100 bp	460 bp
5	65 bp	260 bp
8	45 bp	160 bp
12	20 bp	70 bp
15	15 bp	60 bp
20	12 bp	45 bp

Denaturing Gels		
Polyacrylamide Gel, %	Xylene cyanol FF	Bromophenol blue
5	35 bases	130 bases
6	29 bases	106 bases
8	26 bases	76 bases
10	12 bases	55 bases
20	8 bases	28 bases

Gel Electrophoresis Buffer Solutions

Composition of Gel Electrophoresis Buffers

Buffer	Working Concentration		Stock Concentration (per Liter)		
Tris-acetate (TAE)	1x	20 mM Tris-acetate 1 mM EDTA	20x	Tris base Glacial acetic acid 0.5 M EDTA (pH8.0)	96.9 g 22.84 ml 40 ml
Tris-phosphate (TPE)	1x	90 mM Tris-Phosphate 2 mM EDTA	10x	Tris base 85% Phosphoric acid (1.679g/ml) 0.5 M EDTA (pH8.0)	108 g 15.5 ml 40 ml
Tris-borate (TBE) [A]	0.5x	45 mM Tris-borate 1 mM EDTA	10x	Tris base Boric acid 0.5 M EDTA (pH8.0)	108 g 55 g 40 ml
Alkaline [B]	1x	50 mM NaOH 1 mM EDTA	1x	10N NaOH 0.5 M EDTA (pH8.0)	5 ml 2 ml
Tris-Glycine-SDS Running Buffer [*]	1x	25 mM Tris base 192 mM Glycine 0.1 % SDS	10x	Tris base Glycine 10% SDS	30.3 g 144.1 g 100 ml
Tris-Glycine Running Buffer [*]	1x	25 mM Tris base 192 mM Glycine	10x	Tris base Glycine	30.3 g 144.1 g
Tris-Tricine-SDS Running Buffer [*]	1x	100 mM Tris base 100 mM Tricine 0.1 % SDS	10x	Tris base Tricine 10 % SDS	30.3 g 179.2 g 50 ml

[A] : Precipitation often occurs after long-term storage of high-concentrated 10x TBE. To avoid this problem, prepare a 5x TBE solution and store it at room temperature.
- 1x TBE solution has been originally used in agarose gel electrophoresis. The buffering capacity of 0.5x TBE is good enough for this purpose and it is widely used these days.
- The use of 1x TBE is recommended for polyacrylamide gel electrophoresis. The buffer reservoirs for vertical polyacrylamide gel electrophoresis are relatively small in comparison to those for horizontal agarose gel, and the electric current through the thin slab gel is relatively high, generating heat if the buffer has low concentrations of electrolytes. For this reason, it is necessary to use 1x TBE in polyacrylamide gel electrophoresis.

[B] : Alkaline gel electrophoresis buffer solution should be freshly prepared everytime prior to use.

[*] : Use Tris-Glycine-SDS , Tris-Glycine , and Tris-Tricine-SDS Running Buffer for SDS-polyacrylamide gel

Composition of Gel Loading Buffers

Buffer	6x Gel Loading Buffer	Storage Temp
I	0.25% Bromophenol blue 0.25% Xylene xyanol FF 40% (w/v) sucrose in water	Room temp.
II	0.25% Bromophenol blue 0.25% Xylene xyanol FF 15% (w/v) Ficoll 400 in water	Room temp.
III	0.25% Bromophenol blue 0.25% Xylene xyanol FF 30% (w/v) Glycerol in water	Room temp.
IV	0.25% Bromophenol blue 40% (w/v) sucrose in water	Room temp.
V	Alkaline loading buffer 300 mM NaOH 6 mM EDTA 18% (w/v) Ficoll 400 in water 0.15% Bromocresol green 0.25% Xylene xyanol FF	Room temp.

Glycerol in the gel loading buffer increases the density of a sample, which helps the DNA sample sink into wells formed in a gel, preventing diffusion. Furthermore, dyes present in the buffer gives color to the sample, thereby making it easy to load the sample onto a gel. The dyes in the gel loading buffer solution move toward anode in the same direction of sample DNA to be analyzed in electrophoresis. In commonly used agarose gels, bromophenol blue migrates 2.2 times faster than xylene cyanol FF. Bromophenol blue comigrates with a 300 bp DNA fragment in 0.5x TBE buffer, while xylene cyanol FF with a 4 kbp DNA fragment. The relative migration rates of the two dyes remain constant in agarose gels of 0.5% to 1.4%. The choice of a loading dye is not important, but it is worthwhile to remember that bromocresol blue takes much more vivid blue in alkaline pH than bromophenol blue. Thus, use bromocresol blue in an alkaline gel.

Useful Tips for DNA Electrophoresis

1. Resolution of DNA fragments in an agarose gel is greatly affected by the types of electrophoresis buffers used. TAE buffer (Tris-Acetate-EDTA) is suitable for separation of DNA fragments larger than 4 kbp, whereas TBE (Tris-Borate-EDTA) is better for separation of DNA fragments ranging from 0.1 to 3 kbp. If high voltage (>150 V) is used for faster electrophoresis, it is recommended to use TBE buffer.
2. If excess buffer is used for agarose gel electrophoresis, it may interfere with migration of DNA fragments and distort band patterns of DNA fragments.
3. Optimal amounts of DNA samples help to obtain sharp and high-resolution band patterns of DNA fragments.
4. During electrophoresis, high voltage running may cause heat generation, which results in melting of an agarose gel and thus distortion of DNA bands. Cautions should be exercised to maintain the temperature of a gel apparatus below 30℃. If heat generation is expected, perform agarose gel electrophoresis in a cold room.
5. If an agarose gel below 1% is used to separate supercoiled DNA ladder, the concentration of ethidium bromide should be increased to 2 μg/ml. Depending on the extents in supercoiling, a lower concentration of ethidium bromide would result in band smearing or appearance of extra bands.
6. The use of a loading buffer containing glycerol will result in “smiling”of DNA bands during electrophoresis in a polyacrylamide gel. This “smiling bands” phenomenon is prominent particularly in TBE buffer.
7. DNA fragments with high AT ratio migrate more slowly than expected for its size in a polyacrylamide gel. This becomes more prominent when electrophoresis is carried out at a low temperature. At higher temperature in an agarose gel, this abnormal migration disappears.
8. The minimal amount of DNA that can be detected in a 3-5 mm thick agarose gel is 1 ng per band with ethidium bromide staining. It is recommended to use DNA concentrations below 50 ng/DNA fragment.