

Electrophoresis is a method used to separate and analyze macromolecules based on their size and charge. Discover our detailed electrophoresis protocol, including the preparation of polyacrylamide gel electrophoresis (PAGE) gels and loading controls.

Electrophoresis can be one dimensional (i.e. one plane of separation) or two dimensional. One dimensional electrophoresis is used for most routine protein and nucleic acid separations. Two dimensional separation of proteins is used for finger-printing, and when properly constructed can be extremely accurate in resolving all of the proteins present within a cell.

Here we will describe techniques for one dimensional electrophoresis. We recommend Gel Electrophoresis of Proteins: A Practical Approach (Hames BD and Rickwood D, 1998. The Practical Approach Series, 3<sup>rd</sup> Edition. Oxford University Press) as a reference for basic understanding of 2D electrophoresis protocols.

## Contents

- Preparation of PAGE gels
- Positive controls
- Molecular weight markers
- Loading samples and running the gel
- Loading controls

## Preparation of PAGE gels

Polyacrylamide gels are formed from the polymerization of two compounds, acrylamide and N,N'-methylenebisacrylamide (bis, for short). Bis is a crosslinking agent for the gels. The polymerization is initiated by the addition of ammonium persulfate (APS) along with either DMAP or TEMED. The gels are neutral, hydrophilic, three-dimensional networks of long hydrocarbons cross-linked by methylene groups.

The separation of molecules within a gel is determined by the relative size of the pores formed within the gel. The pore size of a gel is determined by two factors: the total amount of acrylamide present (designated as %T) and the amount of cross-linker (%C). As the total amount of acrylamide increases, the pore size decreases. With cross-linking, 5%C gives the smallest pore size. Any increase or decrease in %C increases the pore size.

Gels can be purchased ready-made or produced in the laboratory (recipes can be found in laboratory handbooks). The Abcam laboratory uses gels from our Optiblot range. Either way, choose the percentage of your gel carefully as this will determine the rate of migration and degree of separation between proteins.

***The smaller the size of the protein of interest, the higher the percentage of acrylamide/bis. The bigger the size of the protein of interest, the lower the percentage of acrylamide/bis.***

The following is a rough guide for choosing an appropriate gel percentage based on protein size. Gradient gels are also available.

Protein size	Gel acrylamide percentage
4-40 kDa	20%
12-45 kDa	15%

10-70 kDa	12.5%
15-100 kDa	10%
25-200 kDa	8%

***Acrylamide is a potent cumulative neurotoxin: wear gloves at all times.***

Place gels in the electrophoresis tank as instructed by the manufacturer and bathe in migration buffer.

## Positive controls

A positive control lysate can be used to demonstrate that the protocol is efficient and correct and that the antibody recognizes the target protein which may not be present in the experimental samples.

***We strongly recommend the use of a positive control lysate when setting up a new experiment; this will give you immediate confidence in the protocol.***

## Molecular weight markers

A range of molecular weight markers will enable the determination of the protein size (see below) and also to monitor the progress of an electrophoretic run. A range of MW markers are commercially available.

We have the following molecular weight markers:

[Molecular Weight Marker \(ab48854\)](#)

[Prism Protein Ladder \(10–175 kDa\) \(ab115832\)](#)

[Prism Ultra Protein Ladder \(10–180 kDa\) \(ab116027\)](#)

[Prism Ultra Protein Ladder \(10–245 kDa\) \(ab116028\)](#)

[Prism Ultra Protein Ladder \(3.5–245 kDa\) \(ab116029\)](#)

## Loading samples and running the gel

Use special gel loading tips or a micro-syringe to load the complete sample into wells. Take care not to touch the well bottoms with the tip as this will create a distorted band.

Never overfill wells. This could lead to poor data and poorly resolved bands if samples spill into adjacent wells.

Load 20–40 µg total protein per mini-gel well.

The gels should be submerged in migration buffer normally containing SDS, except in native gel electrophoresis.

A standard migration buffer (also called running buffer) for PAGE is 1X Tris-glycine:

25 mM Tris base

190 mM glycine

0.1% SDS

Check the pH; it should be around 8.3.

Run the gel for the recommended time as instructed by the manufacturer; this can vary from machine to machine (1 h to overnight depending on the voltage).

When the dye (the migration front) reaches the bottom of the gel, turn the power off. Proteins will slowly elute from the gel at this point, so do not store the gel; proceed immediately to transfer.

## Loading controls

Loading controls are required to ensure that the lanes in your gel have been evenly loaded with sample, especially when a comparison must be made between the expression levels of a protein in different samples. They are also useful to check for even transfer from the gel to the membrane across the whole gel.

Where even loading or transfer have not occurred, the loading control bands can be used to quantify the protein amounts in each lane. For publication-quality work, use of a loading control is absolutely essential.

Visit our [loading control guide](#).

The following table contains information about common loading controls:

<b>Loading control</b>	<b>Sample type</b>	<b>Molecular weight</b>	<b>Caution</b>
Vinculin	Whole cell	125 kDa	
Cyclophilin	Whole cell	24 kDa	
GAPDH	Whole cell	35 kDa	Some physiological factors, such as hypoxia and diabetes, increase GAPDH expression in certain cell types
Cofilin	Whole cell Nuclear Membrane Cytoskeleton	19 kDa	
Alpha tubulin	Whole cell Cytoskeleton	50 kDa	Tubulin expression may vary according to resistance to antimicrobial and antimiotic drugs (Sangrajang S <i>et al.</i> , 1998; Prasad V <i>et al.</i> , 2000).
Beta tubulin	Whole cell Cytoskeleton	50 kDa	Tubulin expression may vary according to resistance to antimicrobial and antimiotic drugs (Sangrajang S <i>et al.</i> , 1998; Prasad V <i>et al.</i> , 2000).
Actin	Whole cell Cytoskeleton	42 kDa	
Beta actin	Whole cell Cytoskeleton	40 kDa	Not suitable for skeletal muscle samples. Changes in cell-growth conditions and interactions with extracellular matrix components may affect actin protein synthesis (Farmer <i>et al.</i> , 1983).
VDAC1/Porin	Mitochondrial	30 kDa	
COX IV	Mitochondrial	20 kDa	Many proteins run at the same 16 kDa size as COX IV.
HSP60	Mitochondrial Membrane	60 kDa	
Lamin B1	Nuclear	66 kDa	Not suitable for samples where the nuclear envelope is removed.
HDAC1	Nuclear	55 kDa	
YY1	Nuclear	45 kDa	
TBP	Nuclear	35 kDa	Not suitable for samples where DNA is removed.
PCNA	Nuclear	30 kDa	
Cdk4	Nuclear Membrane	34 kDa	
Na-K ATPase	Membrane	110 kDa	
Transferrin	Serum	75 kDa	