US/EG Bulletin 2123

# Glossary of Electrophoresis Terms

%C – See crosslinker concentration.

%T – See monomer concentration.

2-D Electrophoresis – An electrophoresis technique in which samples are separated by both isoelectric focusing and SDS-PAGE.

Acrylamide – The monomer used with a crosslinker to form the matrix used for separating proteins or small DNA molecules.

Agarose – A compound derived from seaweed used to form electrophoresis gels for separating DNA or very large proteins. (It is a linear polysaccharide of galactose and 3,6 anhydrogalactose.)

Ammonium Persulfate (APS) – An initiator which generates free radicals used in acrylamide polymerization.

Amperes, Amps – A measure of current. Current in electrophoresis applications is typically measured in milliamps (mA).

Ampholytes – Small molecules which have a large buffering capacity at their isoelectric point (pI). Ampholytes are used to create and maintain a pH gradient in isoelectric focusing.

Anion – A negatively charged ion.

Anode – A positively charged electrode which attracts negative ions (anions). Typically, the anode is marked with red or a '+' sign.

APS – See Ammonium Persulfate.

BAC – (N, N'-bis-acrylylcystamine) A crosslinker used with acrylamide. It has a disulfide bond, so the gel can be dissolved with disulfide-reducing agents.

Band – In a slab gel with wells, the collection of molecules that are moving together, forming a short, horizontal line. The sample migrates out of the well into a lane and separates into bands.

Bis or Bis-acrylamide – (N, N'-methylene-bis-acrylamide) A common crosslinker used with acrylamide to form a support matrix.

Blotting – The transfer of a biomolecule from an electrophoresis gel to a synthetic membrane that binds that biomolecule. The bound biomoleculers are probed while on the surfaces of the membranes. Northern blotting refers to blotting with RNA, Southern blotting refers to blotting with DNA, and Western blotting refers to blotting with proteins.

BME – (2-mercaptoethanol, beta-mercaptoethanol) A reagent used to reduce disulfide bonds. When used in a sample buffer, the reducing reaction using BME will go to equilibrium (the BME will not be used up). It is used in excess (usually at a 5% concentration) so the proteins are completely reduced. BME must be used fresh. See DTT.

Cathode – A negatively charged electrode which attracts positive ions (cations). Typically, the anode is marked with black or a '-' sign.

Cation – A positively charged ion.

Chaotropic Agent – A chemical which disrupts inter- and intra-molecular bonds. Detergents and high concentrations of salt are examples of chaotropic agents.

Charge – The charge of a biomolecule is the sum total of all positive and negative charges on the molecule and is affected by the difference between the pI of the protein and the pH of its environment. If the pH is less than the pI of the molecule, then the molecule has a positive charge and is a cation (and will move toward the cathode). If the pH is greater than the pI of the molecule, then the molecule has a negative charge and is an anion (and will move toward the anode).

Comb – An object used to cast wells in an agarose or acrylamide gel. Square-bottom combs are inserted into the gel sandwich before polymerization to form square-bottomed wells. Sharkstooth combs have triangular teeth and are placed on top of the gel after polymerization. The points of the comb teeth separate the samples from each other. Sharkstooth combs are typically used in sequencing gels.

Continuous Buffer System – An electrophoresis gel system that uses a single buffer throughout the system to separate the sample, as opposed to a discontinuous system, which uses different buffers and sometimes different gel compositions in different parts of the system to focus and separate the components of a sample. The bands in a continuous gel will be as wide as the sample is high in the sample well.

Crosslinker – A molecule used to link polymerizing monomer molecules together to form the gel, a netlike structure. The

holes in the nets are called the pores, and the pore size is determined in part by the crosslinker concentration. The pores may or may not sieve the macromolecules. Bis is a commonly used crosslinker.

Crosslinker Concentration (%C) – The percentage of monomer that is crosslinker. (For example, in bis/acrylamide gels, the grams of bis divided by the total grams of bis plus grams of acrylamide) For a given monomer concentration, there is an optimal crosslinker concentration which gives the smallest pore size, and increasing or decreasing the crosslinker concentration away from the optimum will result in larger pores. Both crosslinker concentration (%C) and monomer concentration (%T) are required to specify the pore size of a gel.

Current – The measure of ion flow in an electrical circuit (the electrophoresis cell and power supply). Current is measured in amperes (A) or milliampreres (mA), and typically represented by 'I.'

Degassing – The removal of dissolved gases from a liquid. For electrophoresis, this is usually accomplished by stirring the liquid under a vacuum. Dissolved oxygen will inhibit acrylamide polymerization.

Denatured – Not in the fully folded conformation found in the native state. The term is typically applied to macromolecules, especially proteins and nucleic acids.

Detergent – A reagent that makes hydrophobic molecules (fats, etc.) soluble in water. Typically, detergents will have a hydrophobic and a hydrophilic moiety. They are usually used in protein electrophoresis to solubilize protein samples.

Diffusion – The random movement of molecules from a region of high concentration to a region of low concentration. Diffusion will cause a loss of resolution, and is increased by increased heat.

Discontinuous Buffer System – An electrophoresis gel system that uses different buffers and sometimes different gel compositions in different parts of the system to focus and separate the components of a sample. Discontinuous systems will typically focus the proteins into tighter bands than in continuous gel systems, allowing larger protein loads. However, the increased focusing may cause proteins in native systems to precipitate out of solution.

Disulfide Bond – A chemical bond joining two sulfur atoms in a large molecule. Reducing a disulfide bond breaks it. Disulfide bonds are commonly found in proteins and contribute to their secondary and tertirary structure.

DTT – (Dithiothreitol) A common reagent used to reduce disulfide bonds. When used in a sample buffer, the reducing reaction using DTT will go to completion (the DTT will be used up). It is used in excess (usually between 5 mM and 15 mM). See BME.

Electro-elution – The use of electrophoresis to move molecules out of a gel. It is commonly used as a method of harvesting the proteins after an electrophoretic separation.

Electroendosmosis – The mass movement of water against the direction of movement of the molecules being separated by electrophoresis.

Electrophoresis – A method of separating biomolecules by the differences in relative mobility in an electrical field.

Elute, elution – To move molecules out of a gel.

Endothermic Reaction – A chemical reaction that absorbs heat. (For example, dissolving urea in water is an endothermic reaction.)

Exothermic Reaction – A chemical reaction that gives off heat. (For example, acrylamide polymerization is an exothermic reaction.)

FIGE – (Field Inversion Gel Electrophoresis) A type of electrophoresis used to separate large DNA fragments by periodically reversing the direction of the electrical field. FIGE works well with fragments up to the size of 200 kilobase pairs.

Fixing a Gel – The precipitation of molecules in a gel to prevent diffusion and loss of resolution.

Free Radical – A reactive molecule with a free electron. Free radicals are required in the polymerization of acrylamide.

Gel – The medium used to separate and stabilize the separation of biomolecules in electrophoresis. In SDS-PAGE, the gel is used as a sieving matrix.

Glycerol – A small nonionic molecule. In vertical gel electrophoresis, glycerol is used to increase the density of the sample buffer so that it sinks to the bottom of the sample well. It is also used to help keep proteins soluble, especially in iso-electric focusing.

Gradient Gel – A gel with gradually changing monomer concentration (%T) in the direction of migration. In SDS-PAGE, gradients are used to separate wider molecular weight ranges of molecules than can be separated with single-percentage gels.

Immunoelectrophoresis – A family of methods based on the electrophoresis of antigenic proteins into an antibody-containing gel which results in the precipitation of antigen-antibody complexes. Common techniques include rocket immunoelectrophoresis, fused rocket immunoelectrophoresis, 2-D immunoelectrophoresis, and crossed immunoelectrophoresis.

Initiator – A reagent that starts a reaction. (TEMED and APS are used as initiators in polyacrylamide polymerization.)

Ion Front – A group of ions moving together during electrophoresis. Due to their small size, they are not hindered by a sieving matrix and move together primarily because of their charge. The ion front marks the movement of the buffer from the upper buffer reservoir.

Ionic Strength – A measure of the ionic concentration of a solution. The ionic strength of a solution affects its electrical resistance.

Isoelectric Focusing – An electrophoresis technique where the protein components of a sample are separated along a pH gradient according to their isoelectric points (pI). Ampholytes are used to establish and maintain the pH gradient in isoelectric focusing.

Isoelectric Point (pI) – The pH at which a biomolecule has a net charge of zero.

Lane – In a slab gel with wells, the strip of gel with bands from a single sample. The track of the sample proteins as they migrate into a gel to form bands is called a lane.

Matrix – Any medium used in a separation molecules. (This term is more general than gel. See Gel.)

Membrane – The thin, paper-like, synthetic sheet to which biomolecules are transferred during blotting.

Monomer – A unit that makes up a polymer. (Acrylamide is a monomer that is polymerized into polyacrylamide.)

Monomer Concentration (%T) – The w/v percentage of monomer in the gel. (For example, in acrylamide/bis gels, %T is the grams of acrylamide plus the grams of bis divided by volume of total gel in ml) Typically, the higher the monomer concentration, the smaller the pore size. Both crosslinker concentration (%C) and monomer concentration (%T) are required to specify the properties of a gel.

Native – Natural. Not reduced or denatured.

Native-PAGE – (Native Polyacrylamide Gel Electrophoresis) A type of protein electrophoresis in which the proteins are in their native, undenatured state during the separation. Typically used for biomolecules that will lose activity if denatured.

Net Charge – The sum total of positive and negative charges on a biomolecule.

Northern Blotting – see Blotting.

Ohm – A unit of resistance.

Ohm's Law – The relationship between voltage (V), current (I), and resistance (R), V = IR.

Overlay Buffer – A buffer put on top of the sample in a well. Also refers to a buffer used on top of acrylamide during polymerization.

PDA – (Piperazine diacrylamide) A crosslinker used with acrylamide. It gives the gel more mechanical strength than other crosslinkers, gives less background with silver staining, and prevents the formation of urea crystals when urea-containing gels are cooled.

pI – See Isoelectric Point.

Plug – In DNA pulsed field electrophoresis, the block of agarose in which the DNA samples are prepared. Preparing the DNA in a plug reduces the chance of shearing the DNA into smaller pieces.

Polymer – A molecule consisting essentially of repeating sub-units.

Polymerization – A chemical reaction where small molecules combine to form larger molecules that contain repeating structural units comprised of the original molecules.

Power – The work performed by an electrical current per unit of time (P = W/t). It equals the voltage times the current (P=VI), and is measured in watts. Power affects heat generation in an electrophoresis system.

Pulsed Field Electrophoresis – A method of DNA electrophoresis that involves periodically changing the direction and strength of the applied electrical field, typically used for DNA molecules larger than 20 kilobases.

Resistance – The degree to which a conductor, such as a wire or electrophoresis buffer, resists the flow of current. Resistance is measured in ohms, and is typically represented by 'R.'

Resolving Gel – The part of a discontinuous electrophoresis gel which separates the different bands from each other.

Rf Value – The relative distance a protein has traveled compared to the distance traveled by the ion front. The Rf value is used to compare proteins in different lanes and even in different gels. It can be used with standards to generate standard curves, from which the molecular weight or pI of an unknown may be determined.

Riboflavin, riboflavin-5'-phosphate – Initiators in acrylamide polymerization that are activated by light. They are used in low pH gels, such as isoelectric focusing gels, since they are effective in acidic solutions. Riboflavin-5'-phosphate is preferred to riboflavin due to its superior solubility.

Running Buffer – The buffer that provides the ions for the electrical current in an electrophoresis run. It may also contain denaturing agents. The running buffer provides the trailing ions in discontinuous electrophoresis.

Sample Buffer – The buffer in which a sample is suspended prior to loading. SDS-PAGE sample buffer typically contains denaturing agents (including reducing agents and SDS), tracking dye, and glycerol.

SDS – (Sodium Dodecylsulfate) A common detergent used in electrophoresis. It binds to proteins at regular intervals giving them consistent (negative) charge-to-mass ratio.

SDS-PAGE – (SDS Polyacrylamide Gel Electrophoresis) A type of electrophoresis used to separate proteins by molecular weight. The proteins are reduced and denatured, and SDS is used to give proteins a consistent charge-to-mass ratio.

Sequencing Gel – An electrophoresis gel used in the determination of the nucleic acid sequence of a fragment of DNA.

Sharkstooth Comb – See Comb.

Southern Blotting – See Blotting.

Spacers – The small blocks set between the two glass plates at the sides of a gel cassette, which create a space between the glass plates in which to pour the slab gel monomer solution.

Stacking Gel – The part of a discontinuous electrophoresis gel which concentrates the components of the sample to create a very thin starting zone. The bands are then separated from each other in the resolving gel.

Standard – A collection of molecules with known properties, such as molecular weight or isoelectric point. Standards are often used to create standard curves, from which the molecular weight or pI of an unknown may be determined.

TEMED – (N, N, N', N'-tetramethylethylenediamine) A catalyst used to activate APS in acrylamide polymerization.

Tracking Dye – A dye used to monitor the movement of molecules through a gel. Bromophenol blue and xylene cyanol FF are common tracking dyes.

Volt – A unit of voltage.

Voltage – The electric potential difference between the anode and the cathode. Voltage is measured in volts, and typically represented by 'V.'

Watt – A unit of electrical power.

Well – The depression in a gel into which the sample is loaded for electrophoresis. The sample migrates out of the well into the gel where it separates into individual bands.

Western Blotting – See Blotting.

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