

Difference Between Horizontal and Vertical Gel Electrophoresis

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Key Difference – Horizontal vs Vertical Gel Electrophoresis

Gel electrophoresis is a laboratory technique used in genetics to separate mixtures containing [DNA](#), [RNA](#), and other [proteins](#) according to their respective charge and molecular size. DNA, RNA or proteins that need to be separated in this method are run through a gel which contains small pores. The molecules are driven through the gel by an electric field. The molecules pass through the pores of the gel, and the speed of the movement is inversely proportional to their respective lengths. Therefore, molecules with lower molecular size will move faster than molecules with a higher molecular weight. The electrical field is generated by the difference in charge at two ends of the gel. One end contains a positive charge, and the other end contains a negative charge. Since DNA and RNA molecules are negatively charged, they will be attracted towards the positively charged end of the gel. Gel electrophoresis can be of two different methods: horizontal gel electrophoresis and vertical gel electrophoresis. **In horizontal gel electrophoresis, the gel is present in a horizontal orientation and is submerged in a continuous running buffer which is present inside the gel box itself. In vertical gel electrophoresis, the buffer system is oriented vertically and is discontinuous with two chambers present on the top and the bottom with [a cathode and an anode](#), respectively.** This is the key difference between horizontal and vertical gel electrophoresis

What is Horizontal Gel Electrophoresis?

Horizontal gel electrophoresis utilizes the basic theory for separation of DNA, RNA or protein molecules according to their respective molecular size and charge. In this technique, the gel is present in a horizontal orientation and is submerged in a buffer which is continuous. [Agarose](#) gel is used to separate the gel box into two compartments. One end of the gel box contains an anode whilst the other end contains a cathode. When a current is applied, the buffer used in this technique allows the creation of a charge gradient. When the charge is applied, the gel tends to heat up. The buffer also functions as a coolant, which maintains the temperature at optimum levels. Recirculation of the running buffer prevents the formation of a pH gradient. A discontinuous buffer system cannot be utilized in horizontal gel electrophoresis since the two compartments

of the gel system get connected with the running buffer. [Acrylamide](#) is used during gel electrophoresis to separate protein mixtures.

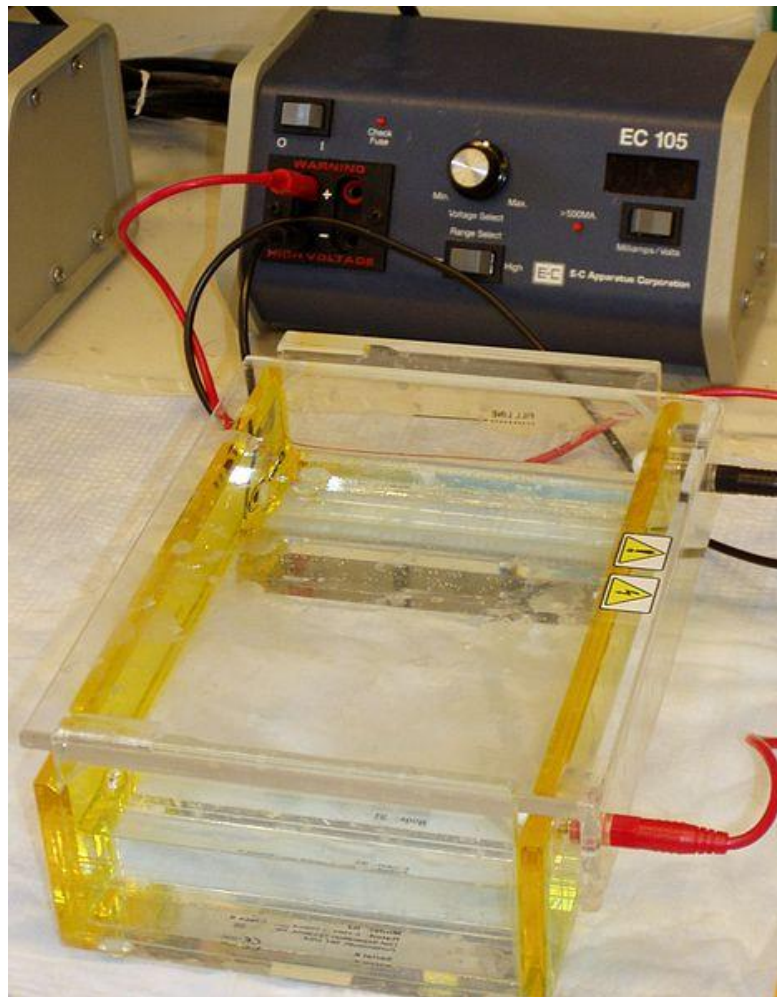


Figure 01: Horizontal Gel Electrophoresis

In horizontal gel electrophoresis, acrylamide cannot be utilized since the gel box is exposed to oxygen. Due to the presence of oxygen, polymerization of acrylamide is inhibited, and this interferes with the formation of the gel. Horizontal gel electrophoresis is an effortless method that is utilized in the separation of DNA and RNA.

What is Vertical Gel Electrophoresis?

Vertical gel electrophoresis technique functions according to the primary theory of gel electrophoresis, but it is considered to be more complex than horizontal gel electrophoresis method. This technique utilizes a discontinuous buffer. A cathode is located in the top chamber, and the anode is located in the bottom chamber. The electrodes present in each compartment provide the required electric field. A thin layer of gel is poured between the two mounted glass plates. Therefore, the top portion of the gel is submerged in the top chamber,

and the bottom portion of the gel is submerged in the chamber at the bottom. Once the current is applied, a small portion of the buffer moves to the bottom chamber from the top chamber through the gel. The current applied in this technique is of minute units. In vertical gel electrophoresis, buffer only flows through the gel. This allows accurate control of gradient of voltage during the separation stage. Acrylamide gel can be utilized since the compartments are not exposed to atmospheric oxygen. Due to the smaller pore size of acrylamide gel, precise separation can be achieved with a higher resolution.

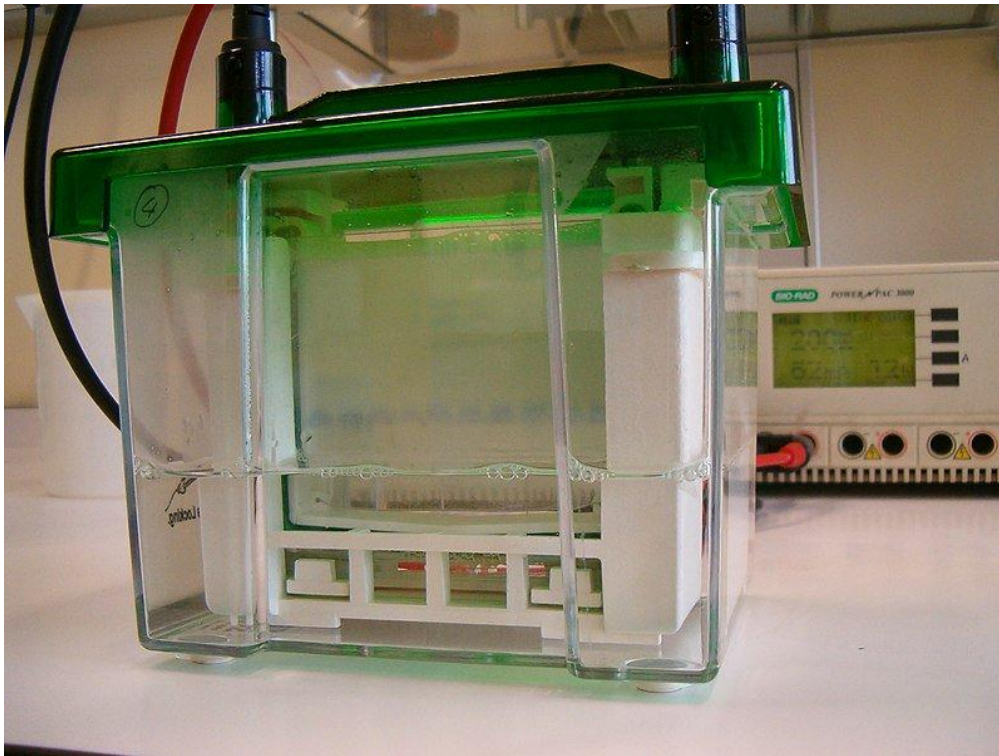


Figure 02: Vertical Gel Electrophoresis

What are the similarities between Horizontal and Vertical Gel Electrophoresis?

- Both systems function according to the basic theory of gel electrophoresis.
- Anode and cathode are used to provide the required electric field in both systems.

What is the difference between Horizontal and Vertical Gel Electrophoresis?

Horizontal vs Vertical Gel Electrophoresis

Horizontal Gel Electrophoresis is a gel electrophoresis technique in which the

Vertical Gel Electrophoresis is a gel electrophoresis technique in which the gel is

gel is present in a horizontal orientation.	oriented vertically.
Buffer	
Horizontal gel electrophoresis consists of a continuous buffer.	The running buffer is discontinuous in vertical gel electrophoresis.
Use of Acrylamide	
Acrylamide cannot be utilized for horizontal gel electrophoresis since the gel box is exposed to atmospheric oxygen.	Since the gel is not exposed to atmospheric oxygen due to two separate chambers, acrylamide could be utilized for vertical gel electrophoresis.
Function	
Horizontal gel electrophoresis is more often used for the separation of DNA and RNA mixtures but not proteins.	Vertical gel electrophoresis is used to separate mixtures of protein.

Summary – Horizontal vs Vertical Gel Electrophoresis

Gel electrophoresis is laboratory technique that is widely used in the separation of mixtures containing molecules of DNA, RNA, and proteins. There are two methods of gel electrophoresis: horizontal and vertical gel electrophoresis. In horizontal gel electrophoresis, the running buffer is continuous whilst in vertical gel electrophoresis it's discontinuous. This is the difference between horizontal and vertical gel electrophoresis. Both systems function according to the common principle of gel electrophoresis.

References:

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2. "Horizontal and Vertical Gel Systems – The Horizontal Gel System." National Diagnostics, [Available here](#). Accessed 28 Aug. 2017.

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APA: Difference Between Horizontal and Vertical Gel Electrophoresis. (2017, September 01). Retrieved (date), from <http://differencebetween.com/difference-between-horizontal-and-vs-vertical-gel-electrophoresis/>

MLA: "Difference Between Horizontal and Vertical Gel Electrophoresis." *Difference Between.Com*. 01 September 2017. Web.

Chicago: "Difference Between Horizontal and Vertical Gel Electrophoresis." *Difference Between.Com*. <http://differencebetween.com/difference-between-horizontal-and-vs-vertical-gel-electrophoresis/> accessed (accessed [date]).



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