D-PAS Staining Solution (Starch Enzyme Digestion Method)

Cat #: A-CSH282

Size: 5×50mL, 5×100mL

Storage: 2-8 °C, Avoid exposure to light (6 months)

Product Description

Component	5×50mL	5×100mL	Store at
Reagent (A): Amylase solution	50ml	100ml	2-8°C
Reagent (B): Periodic acid solution	50ml	100ml	2-8 °C, Avoid exposure to light
Reagent (C): Schiff reagent	50ml	100ml	2-8 °C, Avoid exposure to light
Reagent (D): Mayer's hematoxylin staining solution	50ml	100ml	2-8 °C, Avoid exposure to light
Reagent (E): Acidic ethanol differentiation solution	50ml	100ml	10-30°C

Glycogen staining is a routine staining method in pathology. In 1946, McManus first used the periodic acid-Schiff (PAS) technique to demonstrate mucins. This method is commonly used to visualize glycogen and other polysaccharides. The PAS staining kit not only stains glycogen but also stains neutral mucinous substances, certain acidic substances, as well as cartilage, pituitary glands, molds, fungi, pigments, amyloid-like substances, and basement membranes. Periodic acid (also known as periodic acid) is a strong oxidizing agent that oxidizes the 1,2-glycol groups in carbohydrates and related substances, converting them into aldehydes. The aldehydes react with Schiff reagent to form a magenta compound, resulting in a purple-red color. However, periodic acid can also oxidize other substances within the cells. Therefore, it is important to carefully select the concentration of periodic acid and the duration of oxidation to ensure controlled oxidation that converts the glycol groups into aldehyde groups without excessive over-oxidation. This is a crucial step in the process. PAS technique is the only method for detecting different types of mucinous substances such as glycogen, mucins, and glycoproteins. However, PAS technique alone cannot distinguish between mucins and glycogen. To differentiate mucinous substances (such as mucins or glycogen), an additional glycogen digestion step is required. In

most cases, α-amylase or maltase can be used to catalyze the hydrolysis of glycogen's glycosidic bonds, forming the water-soluble disaccharide maltose. Before applying the PAS technique, glycogen is removed from tissue sections. Human saliva is considered an effective means of digesting glycogen, but due to safety concerns and the lack of standardized saliva, its use is not recommended.

The characteristic of glycogen D-PAS staining solution is that glycogen is treated with amylase before PAS staining. For glycogen digestion, two identical sections are required. After deparaffinization, one section is treated with a buffer containing amylase, while the other section is only treated with the buffer. Then, both sections are stained using the PAS method. The disappearance of staining after digestion indicates the presence of glycogen.

Usage

1. Prepare two identical sections, deparaffinize with xylene, and rehydrate with a graded ethanol-water series.
2. Treat one section with amylase solution at 37°C for 1 hour. Leave the other section untreated as a control, and immerse it in water for 1 hour.
3. Rinse both sections with running water for 5-10 minutes each.
4. Immerse the sections in periodic acid solution and leave them at room temperature for 5-8 minutes, preferably not exceeding 10 minutes.
5. Rinse once with tap water, followed by two rinses with distilled water.
6. Immerse the sections in Schiff reagent and let them soak in a dark place at room temperature for 10-20 minutes.
7. Rinse with tap water for 10 minutes.
8. Stain the cell nuclei with Mayer's hematoxylin staining solution for 1-2 minutes.
9. (Optional) Differentiate with acidic ethanol differentiation solution for 2-5 seconds.
10. Rinse with tap water for 10-15 minutes, followed by a final rinse with distilled water to restore the blue color.
11. Clear with xylene and mount with neutral mounting medium.

Staining Results

Glycogen, neutral mucins, and salivary mucins	Red-purple color
Various glycoproteins	Red-purple color
Cell nuclei	Blue color
Untreated sections: Bright red or red-purple color for glycogen; amylase-treated sections: Glycogen negative.	

Notes

1. Deparaffinization of the sections should be done in a dry and cool environment to avoid affecting the staining results. It is necessary to use a positive control slide to verify the enzyme activity.
2. The oxidation time with periodic acid should not be excessively long, and the optimal temperature for oxidation is between 18-22°C.
3. Reagents A, B, and C should be stored in a sealed container at 4°C. When using them, avoid excessive exposure to sunlight and air. It is recommended to take them out in advance and let them reach room temperature in a dark place before use.
4. The acidic ethanol differentiation solution should be regularly replaced with fresh solution. The differentiation time should be determined based on the thickness of the sections, the type of tissue, and the freshness of the acidic ethanol differentiation solution. Additionally, the rinsing time with tap water after differentiation should be sufficient.
5. The duration of action in the periodic acid solution and Schiff reagent is crucial and should be determined based on the thickness of the sections, the type of tissue, etc.
6. It is advisable to keep the staining time for the sections as short as possible.

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.