Histochemistry

(Cell biology and Histochemistry)

كود

(practical part)

First semester(الفصل الدراسي الاول)

اعداد

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الرموز المستخدمة

نص للقراءة والدراسة

اسئلة للتفكير والتقييم الذاتي

رابط خارجي

- **رؤية جامعة جنوب الوادي ٢٠١٨ -٢٠٢٣**

التميز في التعليم العالي لبناء تنمية مستدامة في صعيد مصر

- **رسالة جامعة جنوب الوادي ٢٠١٨ -٢٠٢٣**

تسعي جامعة جنوب الوادي الى إعداد الخريجين لممارسة مهنية وبحثية منافسة إقليميا وعالمياً من خلال قدرة مؤسسية وفاعلية تعليمية جاذبة وداعمة تمكن الطلاب من اكتساب مهارات متطورة، وباحثين قادرين على تطوير تخصصاتهم بتقديم بحوث إبداعية وتطبيقية، وتقديم خدمات مجتمعية وبيئية متميزة تسهم في التنمية المستدامة من خلال بناء شراكات استراتيجية فاعلة وتعزيز القيم الوطنية والهوية الثقافية، والتطوير المستمر لبرامج وكليات الجامعة وإداراتها وتأهيلها للاعتماد، ورفع جاهزية وتنافسية الجامعة واستقلال فرعيها والتوظيف الأمثل للموارد.

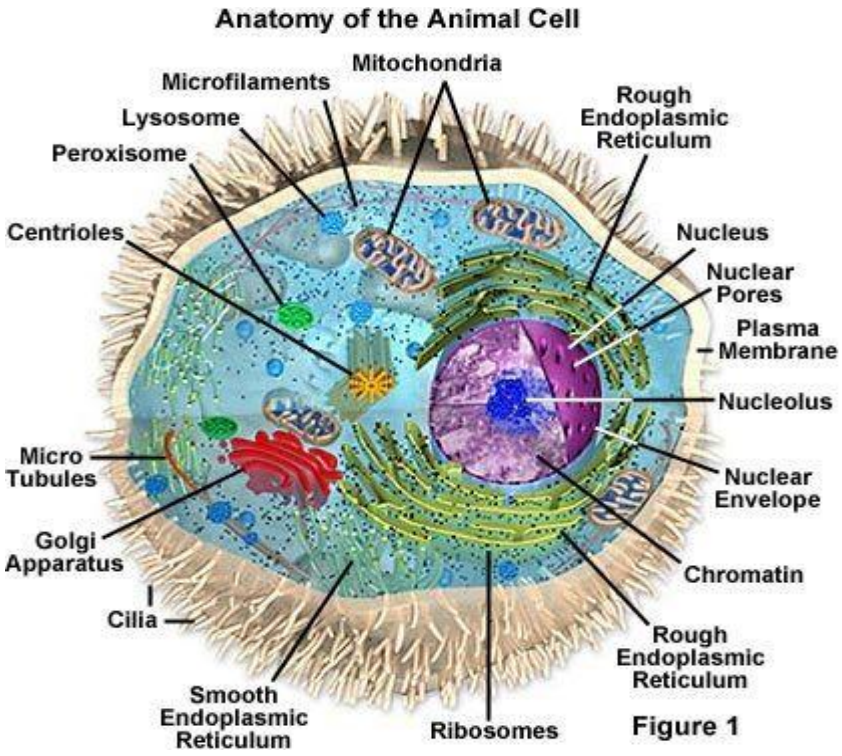
- **رؤية كلية العلوم ٢٠١٨ -٢٠٢٣**

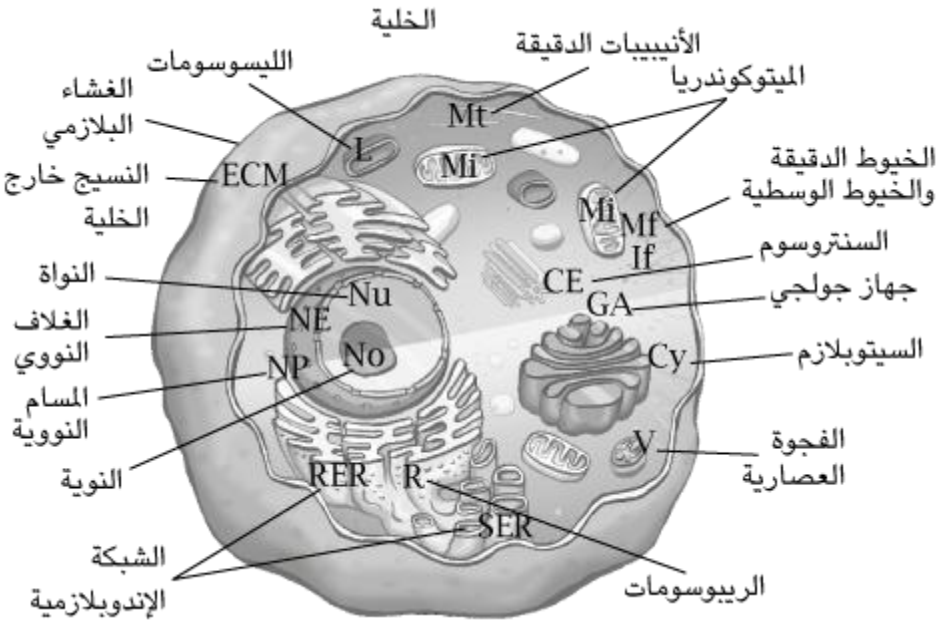
التميز في تعليم العلوم الأساسية والبحث العلمي للمساهمة في التنمية المستدامة

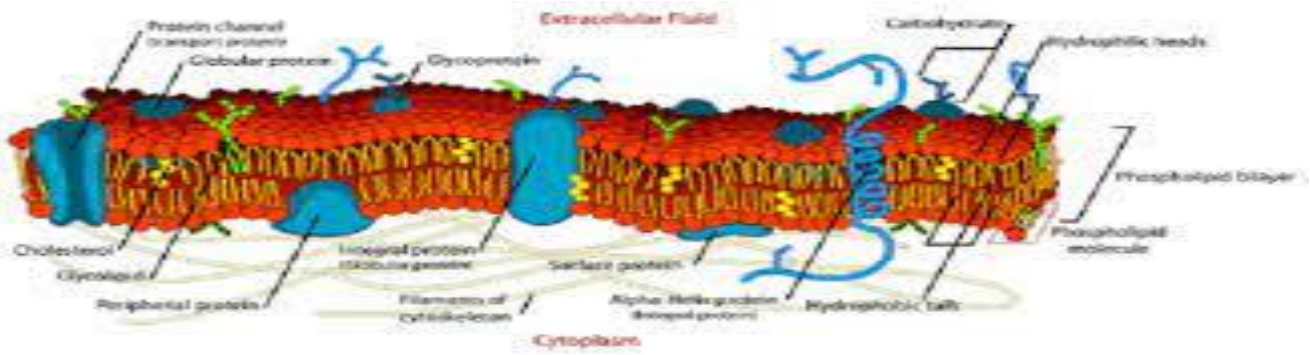
- **رسالة كلية العلوم ٢٠١٨ -٢٠٢٣**

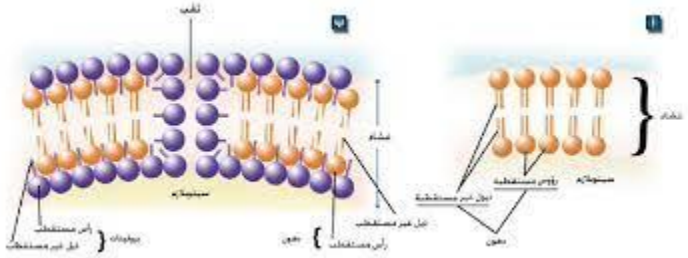
تقديم تعليم مميز في مجالات العلوم الأساسية وإنتاج بحوث علمية تطبيقية للمساهمة في التنمية المستدامة من خلال إعداد خريجين متميزين طبقا للمعايير الأكاديمية القومية، وتطوير مهارات وقدرات الموارد البشرية، وتوفير خدمات مجتمعية وبيئية تلبي طموحات مجتمع جنوب الوادي، وبناء الشراكات المجتمعية الفاعلة.

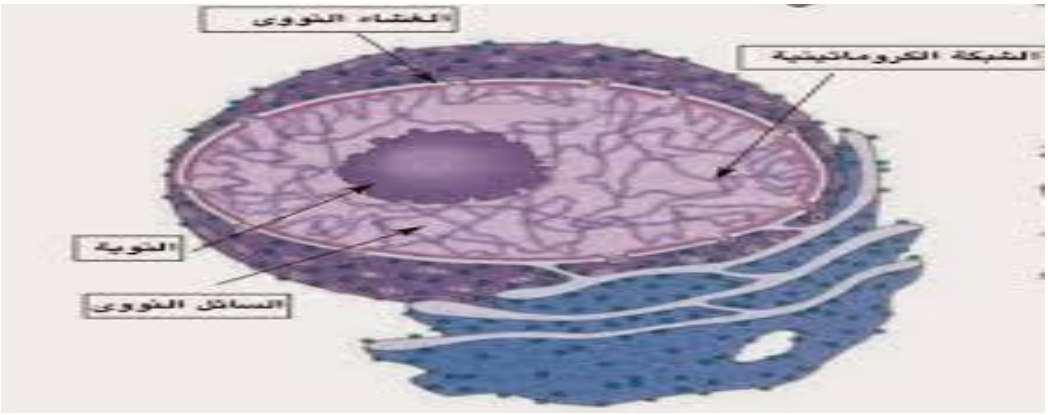
Cell biology

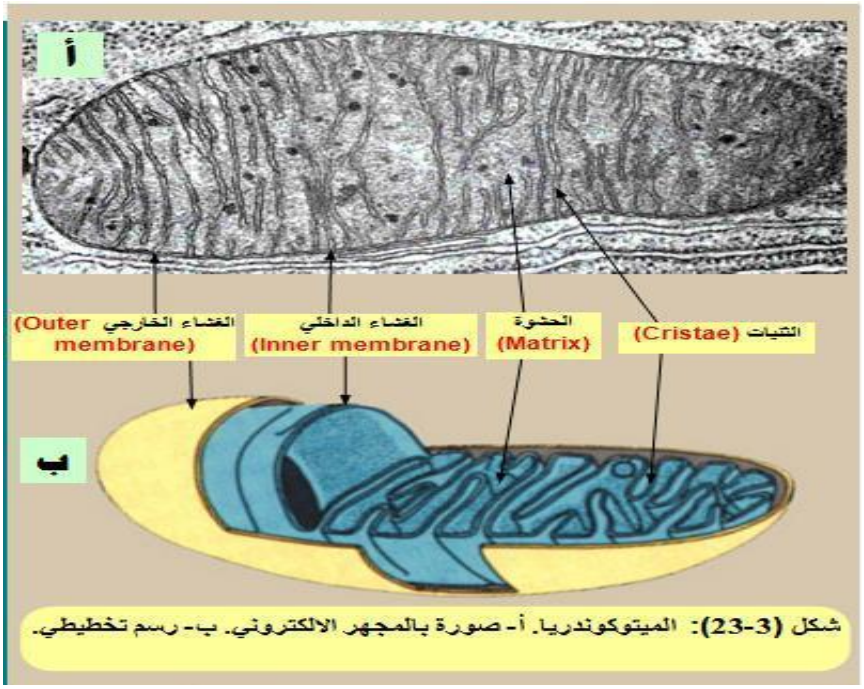




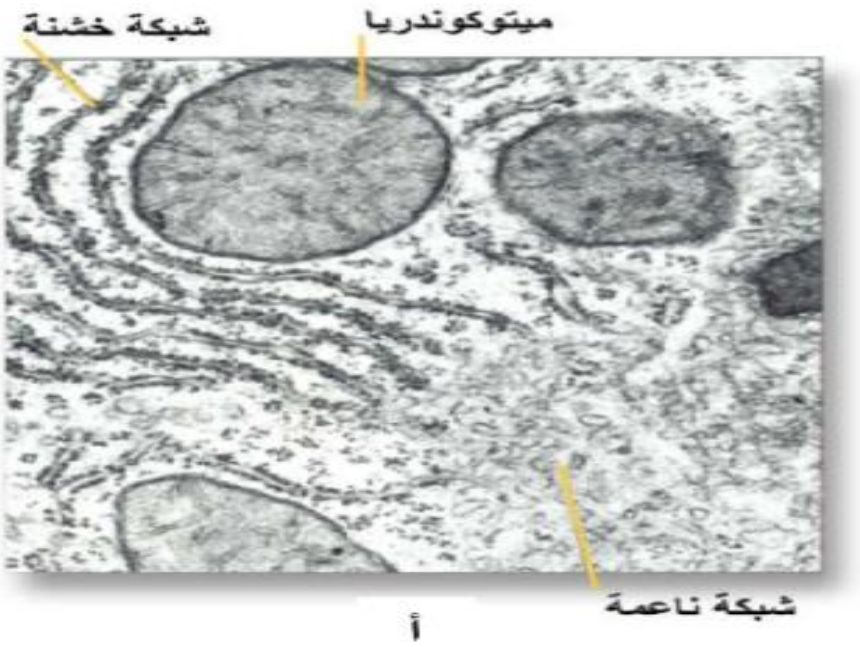

Extracellular Fluid
Protein channel
Globular protein
Glycoprotein
Carbohydrate
Hydrophilic heads
Phospholipid bilayer
Phospholipid molecule
Cholesterol
Glycolipid
Peripheral proteins
Integral proteins
Surface protein
Hydrophobic tails
Cytoplasm







شكل (3-23): الميتوكوندريا. أ- صورة بالمجهر الالكتروني. ب- رسم تخطيطي.


شبكة خشنة
ميتوكوندريا
شبكة ناعمة

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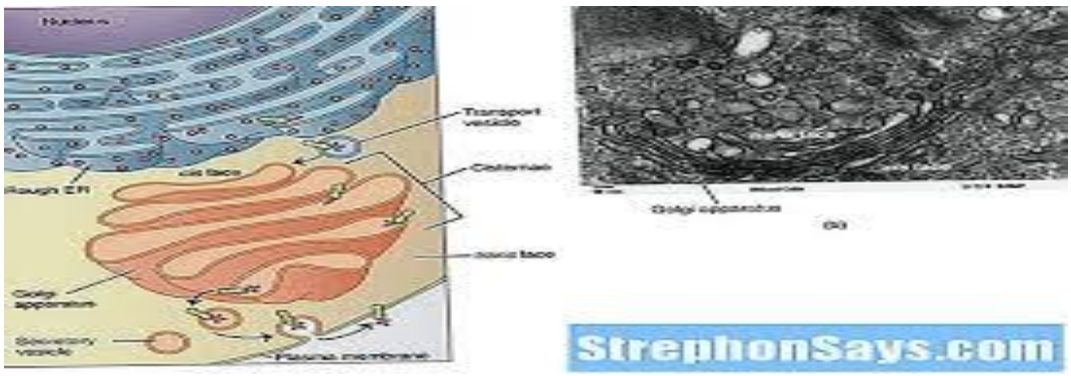

Transport vesicle
Cisternae
Golgi apparatus
Secretory vesicle
Plasma membrane
StrephonSays.com

Histochemistry: is science which studies the patterns of presence and distribution of different

Chemical compounds in tissues and cells of the body, and the role they play in various vital

Activities.

Preparation of tissues for study

There are different methods used, however the basic principles are similar • It usually

Involves hardening of the tissue followed by sectioning (cutting)

- Paraffin technique - Freezing technique- Vital preparation.

1. Paraffin Tissues are hardened by replacing water with paraffin

2. Freezing technique: Water-rich tissues are hardened by freezing and cut frozen

3. Vital preparation is a good method for study the effects of drugs on the living tissues.

The light microscope is not suitable for examining vital specimens. Phase contrast

microscope is used to study the unstained vital preparation.

Protocols followed in Histotechniques

1. Identification & Labeling of the specimen

2. Fixation

3. Dehydration

4. Clearing

5. Impregnation (infiltration)

6. Embedding

7. Section cutting

8. Staining

9. Mounting

Animals should be anesthetized and subjected to cardiac perfusion with saline sol.

Fixation:

This is the process by which the constituents of cells and tissue are fixed in a physical and

chemical state so that they will withstand subsequent treatment with various reagents with

minimum loss of architecture.

This is achieved by exposing the tissue to chemical compounds: fixatives

• Fixatives prevent autolysis and bacterial decomposition and preserves tissue in their natural

state and fix all components

Tissue fixatives

• Buffered formalin (light microscope preparation)

• Buffered gluteraldehyde (electron microscope preparation)

• Osmium tetroxide (electron microscope preparation, preserve and stain)

• Zenker's formal saline

• Bowen's fluid

No fixative will penetrate a piece of tissue thicker than 1 cm

Tissue is sectioned and drop-fixed in a 10% formalin solution (24-48 hours).Formalin has an

acidic nature. Prolonged fixation of samples in formalin causes formalin pigments inside the tissue. This pigments removed by washing sections with alcohol picric acid for some minutes

The excess of formalin can be removed by

Washing samples in running tap water (24 hr.)

Washing samples in 2 changes of chloral hydrate (12 hr. for each) .

The reactions of formalin with tissue are complex because it can combine with a different groups.

Tissue Processing

In order to cut thin sections of the tissues, it should have suitable hardness and consistency when presented to the knife edge.

These properties can be imparted by infiltrating and surrounding the tissue with paraffin

wax, various types of resins or by freezing.

Tissue Processing It can be subdivided into:

a) Dehydration

b) Clearing

c) Impregnation (infiltration)

Types of tissue processing

• There are two types:

1. Manual Tissue Processing

2. Mechanical Tissue Processing.

Manual Tissue Processing

• In this process the tissue is changed from one container of reagent to another by hand

Mechanical Tissue Processing

• In this the tissue is moved from one jar to another by mechanical device

• Timings are controlled by a timer which can be adjusted in respect to hours and minutes|

• Temperature is maintained around 60 ºC .

Dehydration (removal of water) :

It is the process in which the water content in the tissue to be processed is completely

removed by passing the tissue through increasing concentrations of dehydrating agents

Tissues are dehydrated by using increasing strength of alcohol; e.g. 70% (1 hours),80%(1

hours), 90% (1 hours),95% (30 minutes) and 100% (30 minutes).

• Water is replaced by diffusion

During dehydration water in tissue has been replaced by alcohol • the next step alcohol

should be replaced by paraffin wax • as paraffin wax is not alcohol soluble, we replace

alcohol with a substance in which wax is soluble. This step is called clearing.

When samples are dehydrated by Butyl alcohol, the clearing process can be neglected.

Clearing:

• Replacing the dehydrating fluid with a fluid that is totally miscible with both the

dehydrating fluid (alcohol) and the embedding medium (wax). Clearing process is a median

stage between Alcohol and paraffin wax.

Some clearing agents: - Xylene - Toluene - Chloroform – Benzene- Methyl benzoate.

Samples are cleared in 3 changes of Methyl benzoate (24 hr. for each change).

Infiltration (Impregnation):

The tissue is kept in a wax bath containing molten paraffin wax.

Samples are infiltrated in 3 changes of paraffin wax (1 hr for each change).

Embedding:

Embedding: is the process by which impregnated tissues are surrounded by a medium such as

agar, gelatin, or wax which when solidified will provide sufficient external support during

sectioning:

Embedding: It is done by transferring the tissue to a mould filled with molten wax & is

allowed to cool & solidify After solidification, a wax block is obtained which is then

sectioned to obtain ribbons

General Embedding Procedure.

1-Fill the mould with paraffin wax

2-Using warm forceps select the tissue, take care that it does not cool in the air

3-Orienting the tissue in the mould

Orientation Of Tissue In The Block

Correct orientation of tissue in a mould is the most important step in embedding

1. cross section

2. longitudinal section

4-Cool the block on the cold plate.

Sectioning (Section Cutting)

It is the procedure in which the blocks which have been prepared are cut or sectioned and thin

strips of uniform thickness are prepared

The instrument by which this is done is called as a Microtome.

TYPES OF MICROTOMES:

- Rotary microtome
- Freezing (cryostat) microtome
- Ultra microtome

Rotary Microtome
Freezing (Cryostat) microtome

4-Sectioning: Ribbon of sections

Tissue floatation bath

Taking the floating sections onto slide

Adhesives used for fixing the sections on the slides
Albumin solution (Mayor's egg albumin)

It is a thermostatically controlled water bath

It is maintained at a temperature 5 – 6 degrees below the melting point of paraffin wax.

Flattened paraffin sections. Taking the floating sections onto slide. Adhesives used for fixing

the sections on the slides. Albumin solution (Mayor's egg albumin).
*Flattened paraffin sections

Taking the floating sections onto slide

Wax preparation can't be used to demonstrate lipids because

1- Samples are exposed to high temperature.

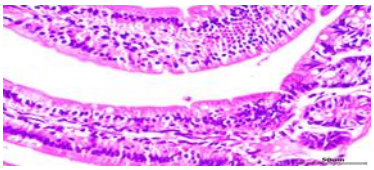
2- Lipid-soluble materials are used during preparation .

So, frozen preparation is a good method for demonstration of lipids and enzymes.

Under the frozen temperature, the enzymes are broken.

Frozen microtome and cryostat is used to cut the frozen samples.

1-Hematoxylin and Eosin



Ionic bonding is the most important type of bonding that occurs in histologic staining

techniques. It involves electrostatic attraction between ions of opposite charge, one of which

is fixed in the tissue, and the second of which is in the dye.

Hematoxylins alone is not technically a dye, and will not directly stain tissues. It therefore

needs to be used in combination with a “mordant” – a compound that helps it link to the

tissue. The mordant used is typically a metal cation, such as aluminium. Haematoxylin in

complex with aluminium salts is cationic and acts as a basic dye. It is positively charged and

can react with negatively charged, basophilic cell components, such as nucleic acids in the

nucleus. These stain blue as a result.

Eosin is anionic and acts as an acidic dye. It is negatively charged and can react with

positively charged, acidophilic components in the tissue, such as amino groups in proteins in

the cytoplasm.

Hematoxylins & eosin staining depends on the basic and acidic properties of tissue

components.

Hematoxylene is a basic dye, acidophilic dye

Eosin is acidic dye, basophilic dye.

The staining procedure for H&E follows a basic protocol:

- Dewaxing
- Dehydration
- Hematoxylin
- Differentiation
- Bluing
- Eosin
- Dehydration
- Clearing
- Cover-slipping

H & E Stain

Intended use

H & E Staining method is used for the routine staining of the cationic and anionic tissue components in tissue sections. This is the standard reference stain used in the study of histochemical tissue pathology.

Summary

Hematoxylin and eosin stain (abbreviated as H & E stain) is one of the principal tissue stains used in routine histology staining methods. It is the most widely used stain in medical diagnosis and is often the gold standard; wherein, when a pathologist looks at a biopsy of a suspected cancer, the histological section is likely to be stained with H & E.

H & E Stain is the combination of two histological stains: Hematoxylin and Eosin. The hematoxylin is a selective nuclear stain which stains the cell nuclei blue, and eosin stains the extracellular matrix and cytoplasm pink, with other structures taking on different shades, hues, and combinations of these colors. The stain shows the general layout and distribution of cells and provides a general overview of a tissue sample's structure. Hence, there is a clear differentiation between the nuclear and cytoplasmic parts of a cell.

Principle

Hematoxylin and eosin are the principle stains used for the demonstration of nucleus and the cytoplasmic inclusions. Alum acts as a mordant and hematoxylin containing alum stains nucleus light blue which turns red in the presence of acid. The cell differentiation is achieved by treating the tissue with acid solution. Counter staining is performed by using eosin solution which imparts pink color to the cytoplasm.

Hematoxylin, a common nuclear stain, is isolated from an extract of logwood (Hematoxylins campechianum). Before hematoxylin can be used as a nuclear stain, it must be oxidized to hematein and combined with a metallic ion (mordant). Most successful mordants have been salts of aluminum or iron. Generally, hematoxylins are classified as progressive or regressive based on dye concentration. Progressive stains (e.g., Mayer’s hematoxylin) have a lower concentration of dye and selectively stain

nuclear chromatin. The desired intensity is a function of time. Regressive stains (e.g., Harris hematoxylin) color all nuclear and cytoplasmic structures intensely. To arrive at correct chromatic response, excess dye must be removed by treatment with dilute acid (differentiation).

Eosin is tetra bromofluorescein (a substituted xanthene), a red acidic dye and fluorochrome. The dye is very soluble in ethyl alcohol and also used for the staining of cytoplasm. Eosin Y is the most commonly used counterstain for hematoxylin.

Reagents / Contents

1. Hematoxylin Harris

Hematoxylin 5.0 g

Ammonium/ Potassium Alum 100 g

Mercuric Oxide 2.5 g

Alcohol 95% 50 mL

Distilled Water 1000 mL

Appearance: Maroon purplish solution.

2. Eosin (AQU.) 2%

Eosin-Y 2.0 g

Distilled water 100 mL

Appearance: Dark reddish solution.

Storage and Stability

Store at 15°C-25°C away from bright light. Use before expiry date on label.

Materials required but not provided

Tissue section specimen on clean grease-free glass slide, staining rack, blotting paper, immersion oil, and microscope. Reagents and solutions required but not provided in the Kit such as xylene, a series of descending

and of ascending grades of alcohol, 1% acid alcohol solution, Scott's Tap Water Buffer (Cat. No. 207191390035) and DPX mountant.

Type of Specimen

Histochemical tissues sections obtained from biopsy specimens.

Procedure

1. Sections are deparaffinized (removal of wax) by placing in xylene for 10 - 15 minutes.

2. Rehydrate section by passing in a series of descending grades of alcohol, finally to water.

3. Place in Hematoxylin Harris solution for 8-10 minutes.

4. Rinse in water.

5. Differentiate the slide in a solution 1% acid alcohol for 10 seconds.

6. Rinse in tap water.

7. Blueing (brining the required blue color to section) is done by putting the section in a solution containing Sodium bicarbonate, $MgSO_4$ and saturated solution of Lithium carbonate (Scott’s Tap Water Buffer, Cat. No. 207191390035) for 2-10 minutes.

8. Counter stain with aqueous Eosin (Aqu.) 2% for 1-3 minutes.

9. Rinse in tap water.

10. Section are dehydrated which is done by a series of ascending grades of alcohol and finally clearing in Xylene.

11. Dry the section by pressing on the filter paper.

12. Mount in DPX and observe under microscope, 40X and 100X under oil immersion lens.

Interpretation of Results

The nuclei of cells are stained blue or dark-purple along with a few other tissues, such as keratohyalin granules and calcified material with Hematoxylin. The cytoplasm and some other structures including extracellular matrix such as collagen stains in up to five shades of pink with Eosin. Most of the cytoplasm is eosinophilic and is rendered pink. Red blood cells are stained intensely red. The background of the tissue remains colorless.

Warranty

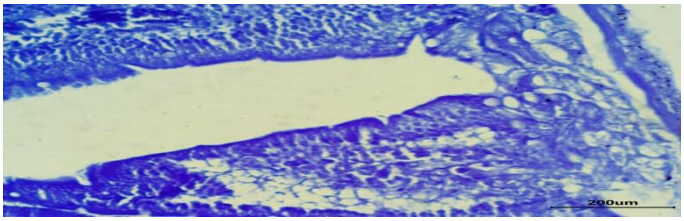
H & E staining solutions are for “In Vitro Diagnostic Use” only. This product is designed to perform as described on the label and pack insert. The manufacturer disclaims any implied warranty of use and sale for any other purpose.

Reference

Data on file: Microxpress®, A Division of Tulip Diagnostics (P) Ltd.

https://l.facebook.com/l.php?u=https%3A%2F%2Fyoutu.be%2FsHeHC_0-BDw%3Ffbclid%3DIwAR0pdzfMyxLOKbdwWpXSlG5In7cN3tDLgx4vxK2Go8_k1vZcYgKzWiaN72s&h=AT08dbg55gJ5eM7tl5nWDgEuk6AYzntHakmYxOmTPochzPV_PB7kOLkjClXzwxwWX1MM4uUc2bkKc7u1y-zvRo_KRoB_r_hZ2QHvG_IarmP7m-EIXGmUm0iCbzXqt2DkP1ZE

2- Demonstration of proteins (Mercury_ Bromophenol blue method):



Proteins are complex, organic compounds composed of many amino acids linked together

through peptide bonds and cross-linked between chains by sulfhydryl bonds, hydrogen bonds

and van der Waals forces.. The proteins in the various animal and plant cells confer on these

tissues their biological specificity.

One of the most important characteristics of the aminoacids in their capacity of combining

with each other to form long chains. This property is due to the presence of the carboxyl

group (-COOH) and the basic amino group (-NH2).

Proteins are amphoteric because it contains the carboxyl group (-COOH) and the basic amino

group (-NH2).

Mechanism of dye

1- Dye deposition

2- Add a weak acid

3- pH<3

4- Protein behaves like a base

The color density of Bromophenol blue stain depends on amount of proteins in tissue

parts.

In some cases, Bromo-phenol stain give red color because it Metachromatic +

Dichromatic stain.

Principle

Mercuric ions of the bromophenol blue solution react with

acidic, sulphydryl and aromatic residues of the protein to give

blue colour.

Fixation and Section

10% neutral buffered formalin; paraffin.

Reagent

Mercuric bromophenol blue : Dissolve 1 gm mercuric chloride

and 0.05 gm. bromophenol blue in 100 ml 2% aqueous

acetic acid.

Method

Bring sections to water. Stain in the mercuric bromophenol

blu2 solution for 2 hours at room temperatue. Rinse sections for

5 minutes in 0.5% acetic acid. Transfer sections dirctly into

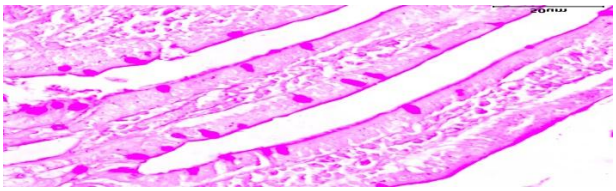
tertiary butyl alcohol. Clear in xylene and mount in DPX.

Result

Proteins—deep blue color

https://l.facebook.com/l.php?u=https%3A%2F%2Fyoutu.be%2FroWDBmUDLUg%3Ffbclid%3DIwAR292x50HyfbE0QaxdN3WAGjQ15KW5aiLzYZBU5jM-5TTot3nm3Sj86kHZc&h=AT08dbg55gJ5eM7tl5nWDgEuk6AYzntHakmYxOmTPochzPV_PB7kOLkjClXzwxwWX1MM4uUc2bkKc7u1y-zvRo_KRoB_r_hZ2QHvG_IarmP7m-EIXGmUm0iCbzXqt2DkP1ZE

Carbohydrates:



Carbohydrates are keto-derivatives or aldehyde –derivatives. Carbohydrates may be

classified as mono saccharides, disaccharides , polysaccharides.

1- Mono saccharides are simple sugars which cannot be hydrolyzed into a simpler form .

2- Disaccharides are the result of condensation of two molecules of mono saccharides

with the loss of one molecule of water.

Maltose is built up of two molecules of glucose. Lactose is built up of glucose and

Gala ctose. Sucrose is formed of glucose and fructose.

3- Poly saccharides are formed by the condensation of many molecules of

Mono saccharides with a corresponding loss of water molcules.

Glycogen is the simple polysaccharide of the animal body. It is often called animal starch.

It is contained in liver cells and muscle fibers.

Sometimes, glycogen is not uniformly distributed in the cytoplasm of fixed cells

particularly in the liver cells where it appears accumulated in certain regions of the cells,

whereas the other regions remain almost empty. This phenomenon is referred to as"

glycogen flight". It is explained as being due to the fact that the fixatives used for the

preservation of glycogen sweep this materials infront of them until it becomes pressed

against the part of the cell membrane opposite to the direction of diffusion.

Glycogen in the liver cells

Liver glycogen has two main source. The first is the glucose carried from alimentary

canal. The second source is the lactic acid produced in the muscle cells as a result of

glycolysis.

To avoid glycogen flight

1- Using frozen preparation method

2- Wash samples after fixation in diluted Osmic acid (2 min).

Description: This method is used for detection of glycogen in tissues such as liver, cardiac and skeletal muscle on formalin-fixed, paraffin-embedded tissue sections, and may be used for frozen sections as well. The glycogen, mucin, and fungi will be stained purple and the nuclei will be stained blue.

Fixation: 10% formalin.

Section: paraffin sections at 5 um.

Solutions and Reagents:

0.5% Periodic Acid Solution:

Periodic acid ---------- 0.5 g

Distilled water ---------- 100 ml

Schiff Reagent: Test for Schiff reagent: Pour 10 ml of 37% formalin into a watch glass. To this add a few drops of the Schiff reagent to be tested. A good Schiff reagent will rapidly turn a red-purple color. A deteriorating schiff reagent will give a delayed reaction and the color produced will be a deep blue-purple.

Mayer's Hematoxylin Solution:

Procedure:

1. Deparaffinize and hydrate to water.
2. Oxidize in 0.5% periodic acid solution for 5 minutes.
3. Rinse in distilled water.
4. Place in Schiff reagent for 15 minutes (Sections become light pink color during this step).
5. Wash in lukewarm tap water for 5 minutes (Immediately sections turn dark pink color).
6. Counterstain in Mayer's hematoxylin for 1 minute.
7. Wash in tap water for 5 minutes.
8. Dehydrate and coverslip using a synthetic mounting medium.

Results:

Glycogen, mucin and some basement membranes --- red/purple

Fungi ---------- red/purple

Background ---------- blue

Demonstration of glycogen (Best's carmine method):

The reason is that complex sugars include a high amount of hydroxyl groups, which gives the

complex sugar molecule an anionic nature (negative ion). As for the dye in all media, it

behaves like a positive ion, and tends to combine strongly with complex sugars by forming strong hydrogen bonds.

Stain procedure.

1 – Digest sections (see above)

2 – Stain in hematoxylin for 10 mins to stain nuclei.

3 – Rinse rapidly in 1% acid alcohol to clear the background.

4 – Wash well in tap water to remove acid alcohol.

5 – Stain in Best's Carmine stain for 10-15 minutes ina Coplin jar.

6 - Wash in Differentiator solution until red stain stops running from the slide (usually takes just a few seconds).

7 - Dehydrate through alcohols and xylene and mount coverslip in DPX.

8- Examine.

Results:

Glycogen Red

Nuclei Blue

https://www.youtube.com/watch?v=QDN0evB5Ca8

Nucleic acids(Feulgen reaction):

PRINCIPLE :

Acid hydrolysis, designed to separate selectively 2 purine bases, namely adenine and guanin, from DNA molecule;staining of apurinic acid resulting from hydrolysis with Schiff reagent. This reagent can be used since free deoxyribose changes to aldehyde in acid environment. Feulgen reaction is highly selective for DNA. Moreover, this reaction allows a very precise localisation of DNA .

PROCEDURE :

1. Bring section to distilled water Put on the section 10 drops of R1, leave to act 10 minutes.

Double washing in distilled water

2. Put on the section 10 drops of R2, leave to act 10 minutes .

3. Drain the slides without washing and put on the section 10 drops of R3 , leave to act 2 minutes .

Wash in runing tap water for 5 min .

4. Counterstain, if required, put on section 10 drops of reagent (4) for

two minutes, then wash with distilled

water .

Dehydrate through ascending alcohols;

clear in xylene and mount

RESULTES :

Nuclear DN : appears reddish purple

Cytoplasm : green .

REFERENCE :

De Tomasi JA. Improving technique of the

Feulgen stain . Stain Technol 1936; 11: 37 .

https://www.youtube.com/watch?v=iSCrTSZotjM