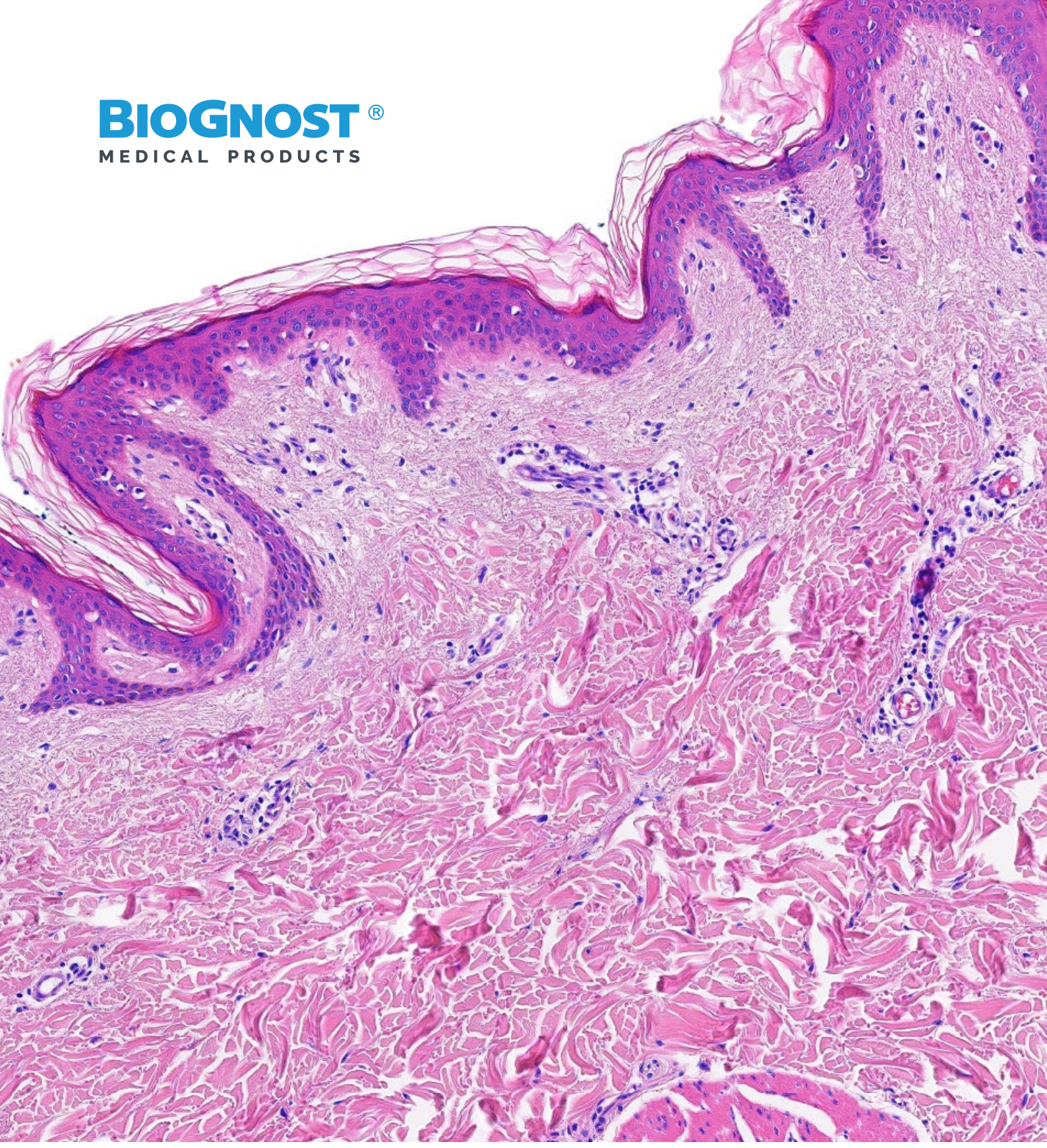
BIOGNOST®
MEDICAL PRODUCTS



H&E

Hematoxylin and Eosin staining

www.biognost.com

Hematoxylin and Eosin staining

Hematoxylin and Eosin (H&E) staining is the oldest and most commonly used staining technique in routine pathology for medical diagnosis. It is of essential importance for diagnosis even today. While other stains and protocols for staining cells and tissue sections have been developed, the original method developed almost 150 years ago remains relatively unchanged. Hematoxylin is not even synthetically produced, but is instead extracted from the logwood tree. Even so, H&E staining remains the most common staining protocol for applications in histology.

However, H&E staining is not standardised. The quality of H&E stains and staining consistency depends on the type of hematoxylin reagent, the protocol used by the lab, and the age of the reagents. Pathologists or diagnosticians have individual preferences for section thickness, intensities, and contrast. The choice of which reagents to use must take into consideration: method of staining (manual vs. automatic), safety, cost, convenience, availability, quality as well as personal preference. For this reason BioGnost offers a range of staining reagents and protocols, developed according to the reference literature and optimized with the aim of constant product improvement. With the right protocol, every lab can get excellent results, enhance nuclear details and emphasize intensity gradations of cytoplasmic staining.

The combination of the hematoxylin and eosin dyes was first used to stain tissues in 1876 by the chemist Wissowzky. Hematoxylin, or more correctly its oxidized form hematein, binds with a mordant (typically Al^{3+}) to stain DNA in cellular matter. It is thought to bind with the negatively charged phosphate groups that comprise the DNA backbone, then undergo complex coordination or conjugation to become a permanent stain of the nucleus. Together with its Al^{3+} mordant, the dye produces a blue color in neutral to basic conditions. Conversely, the anionic eosin Y will bind to positively charged groups on proteins, such as amino groups.

Hematoxylin is colorless when pure. It becomes a dye only after oxidation to hematein. The intense colors of hematoxylin stains are those of complexes of hematein with metal ions. The specific staining properties vary with the metal, the pH of the solution, and the concentrations of the ingredients. In hemalum stains (in German *hemalaun*), the metal is aluminum, more precise potassium alum (KAl). The next most common metal ion used in conjunction with hematoxylin is iron (III), which brings about oxidation to hematein and forms dye–metal complexes that are almost black. Other metal ions that complex with hematein include bismuth, chromium and wolfram.

BioGnost offers 6 different types of Hematoxylin (G1, G2, G3, H, M and ML) with 2 different types of Eosin (alcoholic and aqueous) which bring a clear and consistent staining to your laboratory. The right protocol for every hematoxylin and eosin gives clear, quickly stained slides to facilitate accurate diagnosis. One litre of BioGnost Hematoxylin can stain up to 10,000 slides with the same staining intensity. All reagents used in H&E staining are stabilized and ready to use.

THE ADVANTAGES OF BIOGNOST'S H&E REAGENTS ARE:

- **quality in diagnostics** – different intensity gradations for high quality of nuclear and cytoplasmic stainings
- **optimized protocols** – each protocol optimized specifically for different hematoxylin and eosin reagent
- **improved formulations** – optimized and stabilized solutions with constant improvement
- **variety of solutions** – possibility to choose combinations that best suit specific need
- **ready to use reagents**

There are three types of H&E staining: regressive, modified regressive and a progressive method. The regressive staining method overstains the tissue and then decolorizes the tissue with an acid solution (Hem Diff Strong). The modified regressive method uses weak acid (Hem Diff) for the differentiation of the hematoxylin. The progressive staining stains to a desired intensity without initially overstaining. In a regressive staining method **strong** hematoxylin is used (Hematoxylin H). In a modified regressive staining **moderate** hematoxylins are used: Hematoxylin G2, G3 and ML. In a progressive staining method **delicate** hematoxylins are used such as Hematoxylin G1 and M.

These formulations and protocols provide a variety of colors, potencies and staining patterns. Some stain goblet cells (Gills), others do not (Harris). Gill hematoxylins have three formulations (1, 2 and 3). Gill 1 has a strength which stains the delicate chromatin pattern in cytological preparations but can also be used with histological samples. Gill 2 and 3 are used in tissue staining.

Eosin in the H&E procedure is referred to as a counterstain. It stains nearly everything that hematoxylin does not stain. When applied correctly, eosin produces three different colors which can be used to differentiate various tissue elements: red blood cells stain dark reddish orange, collagen stains a lighter pastel pink and smooth muscle stains bright pink. Half a litre of BioGnost Eosin can stain up to 5,000 slides with the same staining intensity.

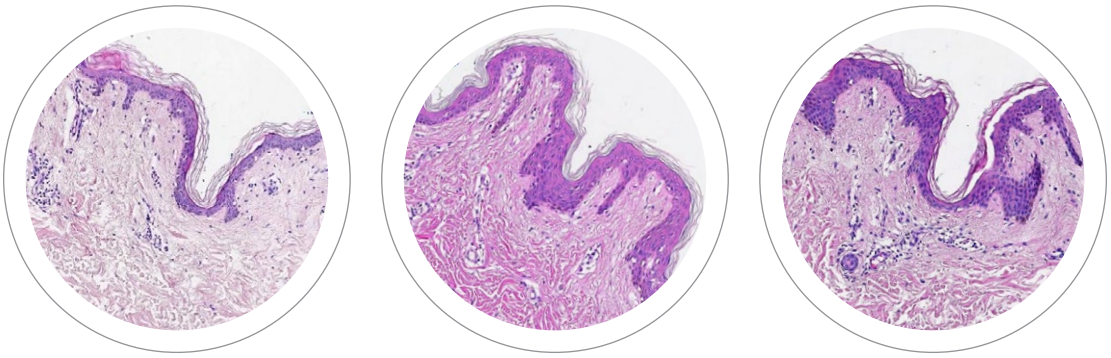
PRINCIPLE OF H&E STAINING:

1. **Deparaffinization** of the sections with BioClear or BioClear New
2. **Rehydration** with Histanol 100, Histanol 95 and Histanol 70
3. **Staining** with Hematoxylin (G1, G2, G3, H, M or ML)
4. **Differentiation** with Hem Diff (for Hematoxylin G2, G3 and ML) or Hem Diff Strong (for Hematoxylin H)
5. **Bluing** with Scott's solution, Bluing reagent or BioBluing Buffer
6. **Rinsing** with tap water
7. **Staining** with Eosin (alcoholic or aqueous)
8. **Dehydration** with Histanol 95 and Histanol 100
9. **Clearing** with BioClear or BioClear New

Staining results

Every pathologist has their own preferences regarding the staining intensity. Some of them prefer the strong intensity and contrast, some of them prefer it more delicate. That is why BioGnost in its portfolio offers a broad selection of reagents, several types of hematoxylin and eosin stains as well as additional reagents such as differentiators, bluing buffers/reagents, alcohols and xylene/xylene substitutes.

There are lots of possible staining results for you to choose what suits you best:



Delicate intensity ●

Moderate intensity ● ●

Strong intensity ● ● ●

Many different factors influence the staining result. Beside the quality of the reagents, it is very important to define the staining procedure. Staining with hematoxylin and eosin reagents is only the middle part of the staining procedure: deparaffinization, differentiation, rinsing, bluing, dehydration, and clearing are also very important parts of the staining protocol. Of course, the staining method (progressive, regressive, or modified regressive method) is critical for getting clear and consistent staining results. Incubation time of every reagent is also very important. It is very interesting how with different types of hematoxylin or eosin you can get the same or different result, depending on the incubation time. The simple and most commonly used staining technique is very tricky and complex so lots of skills are necessary to get the right protocol.

Our H&E reagents and protocols developed at BioGnost R&D are universal, simple and optimized for both automatic and manual staining. We can adjust our protocols for every automatic slide stainer, doesn't matter how many staining tanks the machine has or what other features of the machine are. There are other protocols that we can create but they are tied to different machines and laboratories. For example, on our stainers we didn't include options of shaking or heating the reagents so our protocols are universal and can be applied to any H&E stainer (with or without these functions). With our formulations we can get several hundreds of protocols depending on which type of hematoxylin/eosin is used for staining, which slide stainer is used (with different options) and, of course, what are the preferences of pathologists. It is of significant importance that every lab has the best staining protocol to reduce inadequate results so the pathologist can make a reliable diagnosis.

Hematoxylin reagents

Each of BioGnost Hematoxylin is properly oxidized and specially stabilized in order to maintain the reagent's reactivity for prolonged periods of time, as well as to maintain the quality of the reagent. Every staining provides a crystal clear and highly detailed image of the cell's nucleus, which in turn makes abnormal or pathological changes easier to detect.

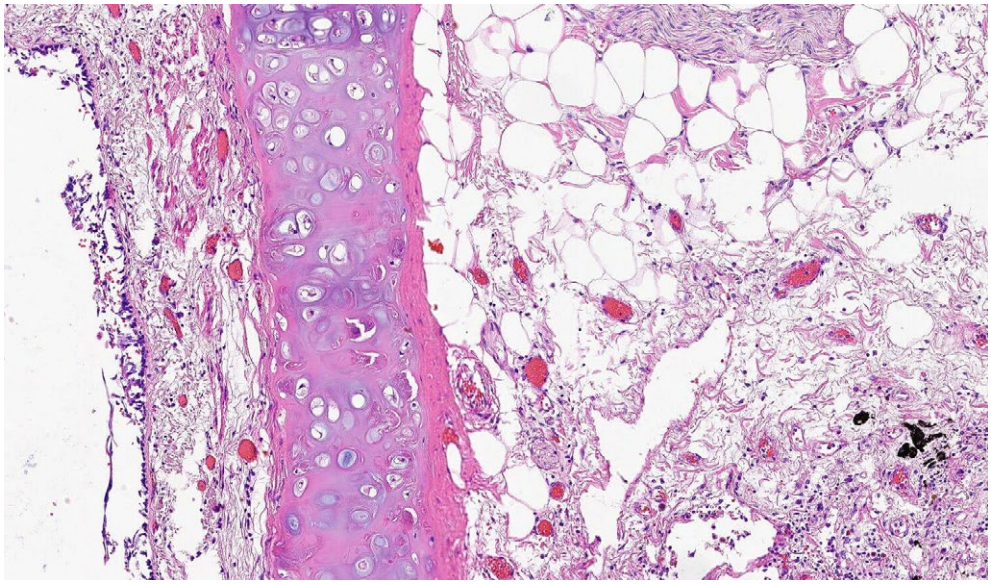
Type of Hematoxylin	Type of staining protocol		
	Progressive method	*Regressive method*	*Modified regressive method*
Hematoxylin G1 ●	√	-	-
Hematoxylin G2 ●●	√	-	√
Hematoxylin G3 ●●●	√	-	√
Hematoxylin H ●●●	√	√	√
Hematoxylin M ●	√	-	-
Hematoxylin ML ●●	√	-	√

Legend: ●●● Strong ●● Moderate ● Delicate

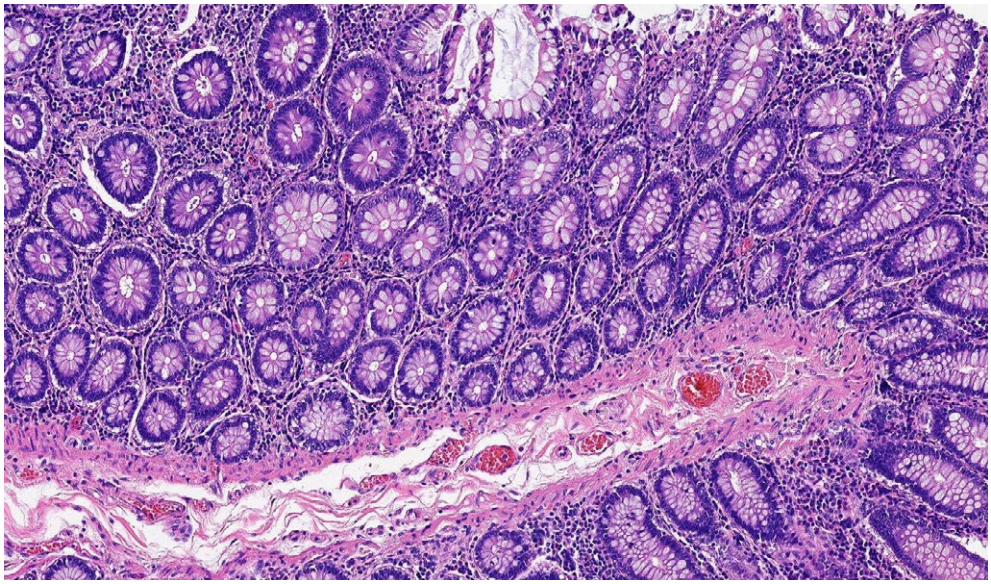
Hematoxylin G1

Hematoxylin G1, according to Gill, is ideal for staining goblet cells. This is a new generation reagent for delicate **progressive** staining in histopathology and cytology.

Check our staining results!



Human lung stained with Hematoxylin G1 and Eosin 0.5% alcoholic (progressive staining method).

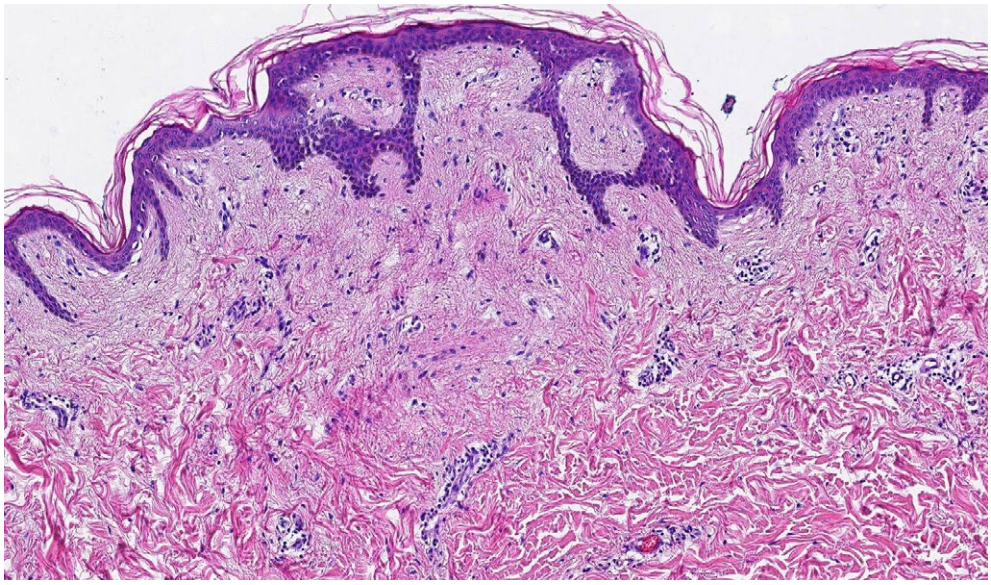


Human intestine stained with Hematoxylin G1 and Eosin 0.5% alcoholic (progressive staining method).

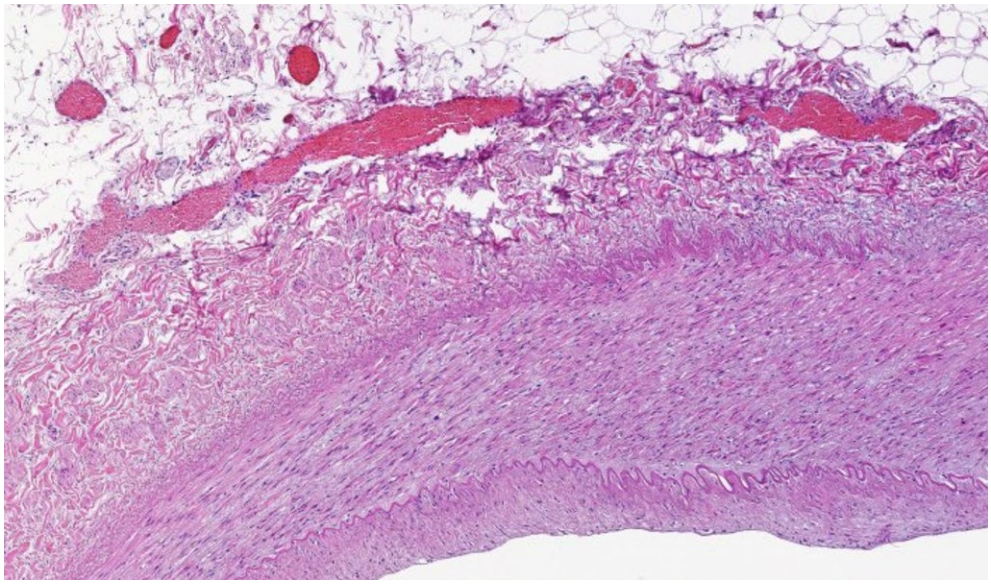
Hematoxylin G2

Hematoxylin G2, according to Gill, contains double the concentration of hematoxylin compared to Hematoxylin G1. This is a moderate intensity new generation reagent for **progressive** and **modified regressive** staining in histopathology, cytology and for counterstaining in immunohistochemistry.

Check our staining results!



Human skin stained with Hematoxylin G2 and Eosin 1% alcoholic (modified regressive staining method).

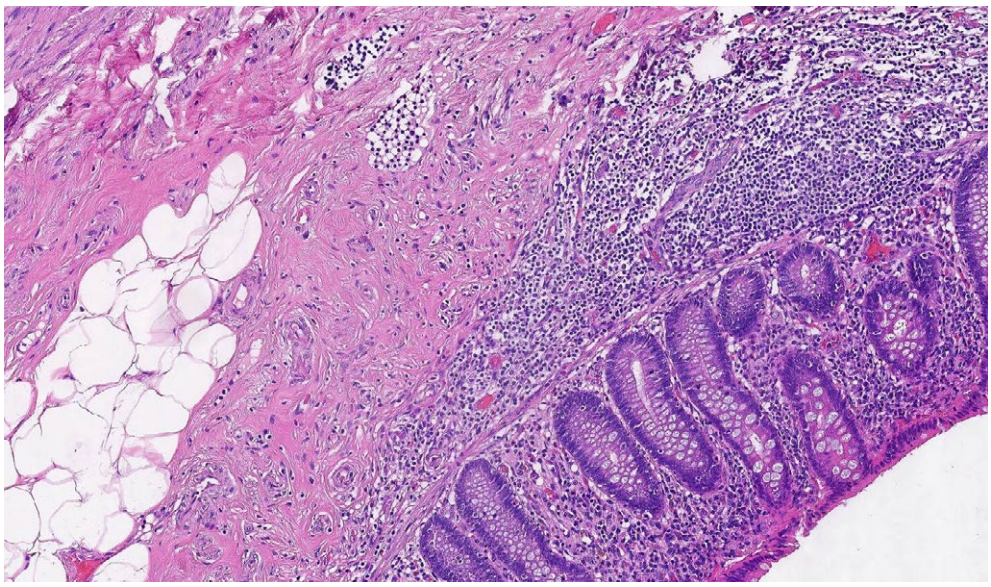


Human artery stained with Hematoxylin G2 and Eosin 1% alcoholic (modified regressive staining method).

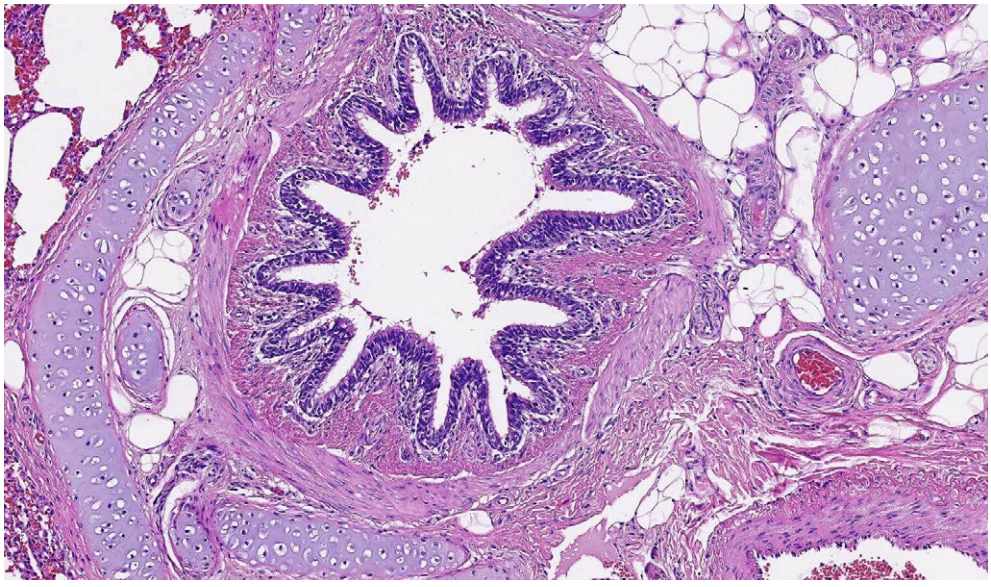
Hematoxylin G3

Modified hematoxylin G3 contains triple the concentration of hematoxylin compared to Hematoxylin G1. This is a strong intensity new generation reagent for **progressive** and **modified regressive** staining in histopathology and cytology.

Check our staining results!



Human appendix stained with Hematoxylin G3 and Eosin 2% aqueous (modified regressive staining method).

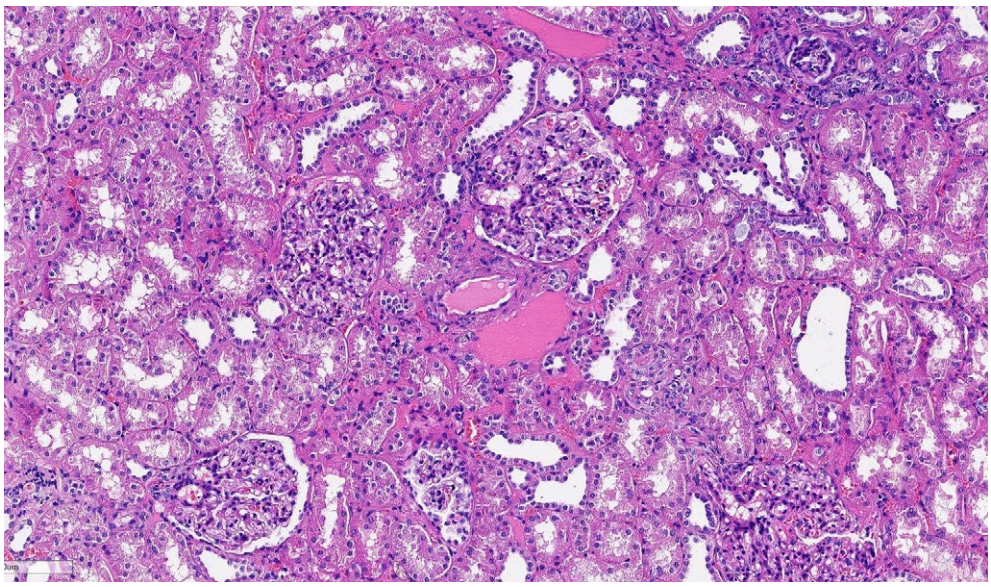


Porcine lung stained with Hematoxylin G3 and Eosin 2% aqueous (modified regressive staining method).

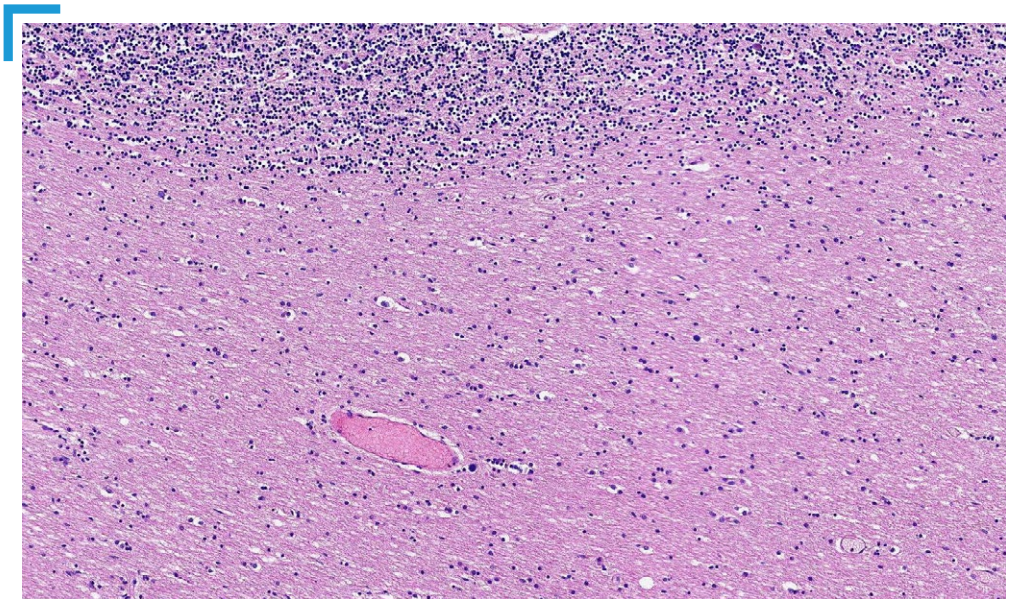
Hematoxylin H

A modified hematoxylin H, according to Harris, is the most commonly used formulation in H&E staining. This is a strong intensity reagent for **progressive**, **regressive** and **modified regressive** staining in histopathology.

Check our staining results!



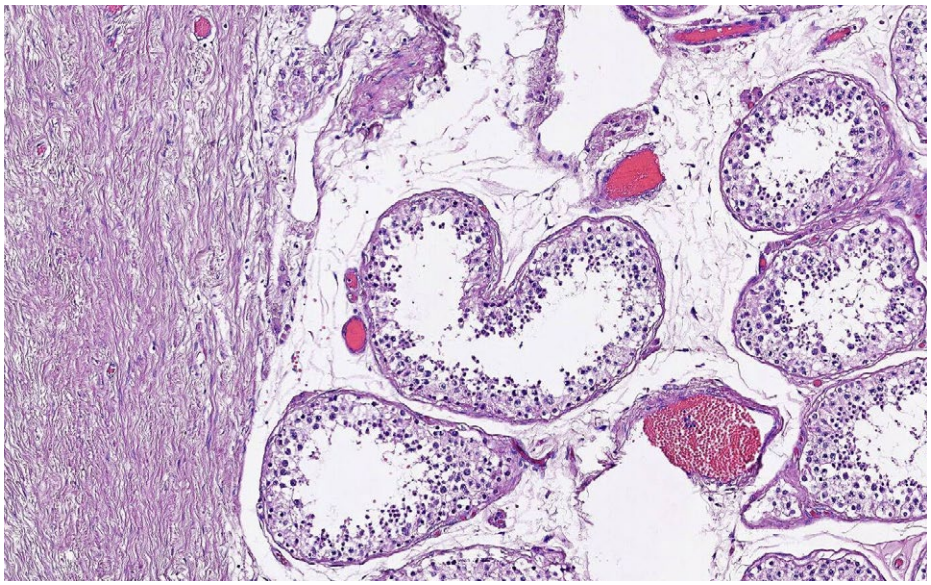
Human kidney stained with Hematoxylin H and Eosin 1% aqueous (regressive staining method).



Human brain stained with Hematoxylin H and Eosin 1% aqueous (regressive staining method).

Hematoxylin M

A modified hematoxylin M, according to Mayer. This is a weaker intensity reagent for **progressive** staining in histopathology.

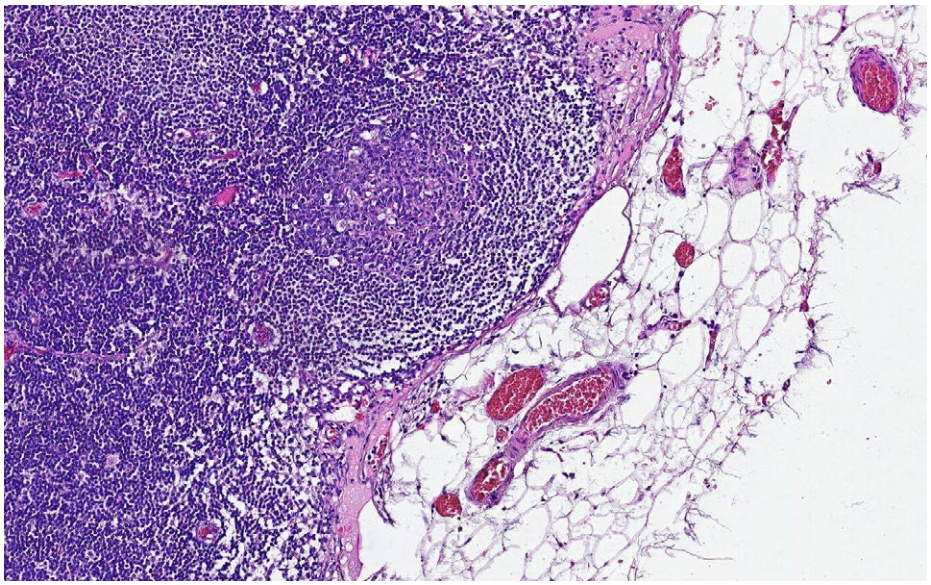


Check our staining results!

Human testis stained with Hematoxylin M and Eosin 1% alcoholic (progressive staining method).

Hematoxylin ML

A modified hematoxylin ML, according to Mayer-Lillie. This is a strong intensity new generation reagent for **progressive** and **modified regressive** staining in histopathology.



Check our staining results!

Human lymph node stained with Hematoxylin ML and Eosin 1% alcoholic (modified regressive staining method).

Differentiation and bluing buffers

NEW

Differentiation of the nucleus enables clear visualization of nuclear structures in regressive and modified regressive protocols, while the bluing buffer turns the hematoxylin and nucleus blue.

HEM Diff

Differentiation reagent based on a weak acid, perfect for the modified regressive staining method. Produces crisp nuclear chromatin detail and eliminates undesirable background staining. Premixed and ready-to-use. Non-hazardous. Used for differentiation of Hematoxylin G2, G3 and ML.

HEM Diff Strong

Strong differentiation reagent based on acid alcohol, perfect for the regressive staining method. It provides excellent differentiation between nuclear and non-nuclear structures. Used for differentiation of Hematoxylin H.

BioBluing Buffer

A buffered alkaline rinse with a mild pH (8.5). Ensures optimal staining and proper cellular color staining. Because of alkaline pH, it turns the hematoxylin blue. BioBluing Buffer is premixed, ready-to-use and tinted blue for easy identification. Utilizes a buffer system to prevent pH fluctuation.

Eosin reagents

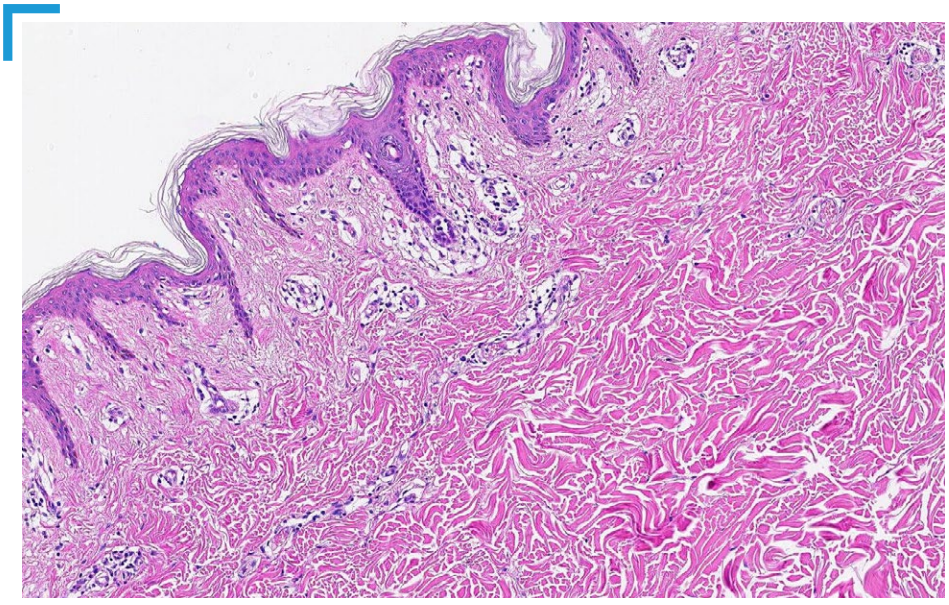
Eosin Y (yellowish eosin) solutions are commonly used as a counterstain to hematoxylin in the standard H&E staining method. Eosin Y is an anionic dye that stains basic cell components (such as cytoplasm, collagen, muscle fibers and erythrocytes) bright red.

Type of Eosin	Concentration of Eosin Y in the solution			
Aqueous	0.2%	0.5%	1%	2%
Alcoholic	/	0.5%	1%	2%

Additional types of alcoholic Eosin:

Eosin Contrast

Modified alcoholic solution for cytoplasmic counterstaining. The reagent contains Eosin Y and Phloxine B for enhanced counterstaining effects.

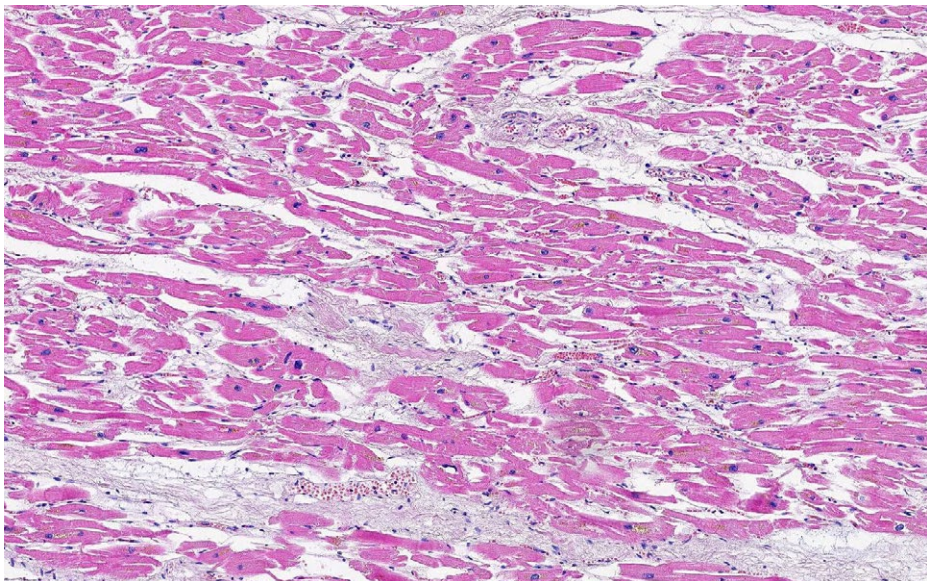


Human skin stained with Hematoxylin G2 and Eosin Contrast (modified regressive staining method).

Check our staining results!

Eosin Contrast PLUS

Modified alcoholic solution for intensive cytoplasmic counterstaining. Reagent contains Eosin Y, Phloxine B and Biebrich Scarlet dyes for additional counterstaining effects.



Human heart muscle stained with Hematoxylin G1 and Eosin Contrast Plus (progressive staining method).

Check our staining results!

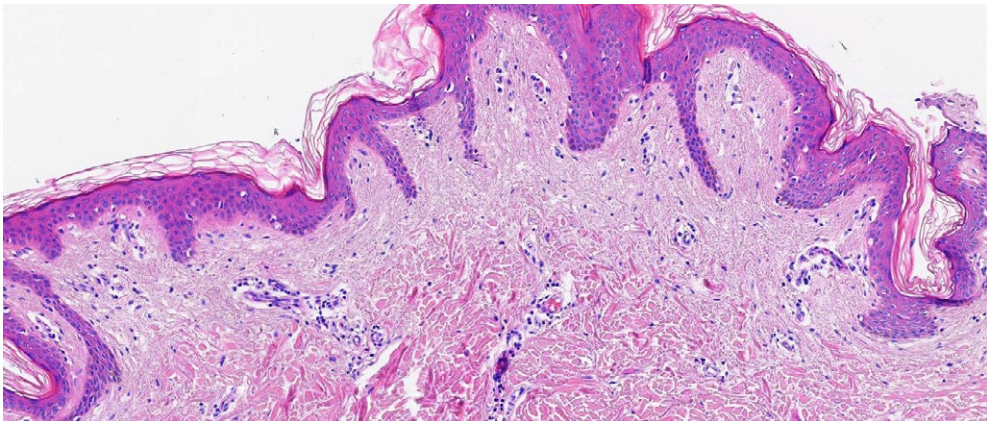
NEW

Check our staining results!

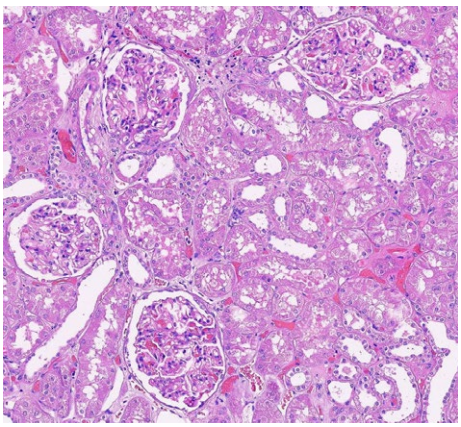
Hematoxylin substitute

ErioGnost reagent

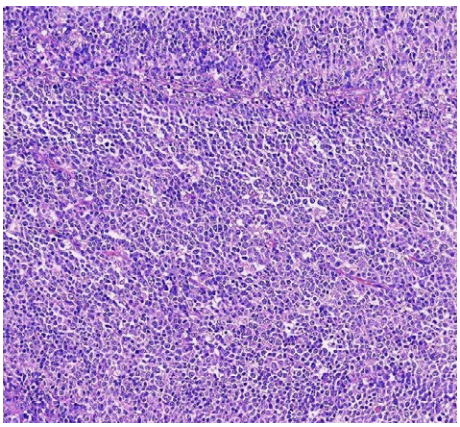
ErioGnost reagent is an acid fast reagent for nuclear staining, comparable to hematoxylin staining. It is eco-friendly synthetic replacement for hematoxylin reagents. Eriochrome Cyanine R belongs to anionic sulfonphthalein mordant dyes. It can be used independently as pH indicator or as red anionic dye; however, it creates intensely stained complexes with transition metal ions (such as iron ions), and because of that this dye is most commonly used in histology as hematoxylin substitute. Eriochrome represents economic and ecologically acceptable synthetic replacement for hematoxylin, and its working solutions show superior stability compared to hematoxylin working solutions. Owing to the technical and commercial complexities of hematoxylin production and distribution, repeated shortages of hematoxylin could occurre, because hematoxylin is a natural dye, extracted from the heartwood of a subtropical tree (logwood tree, *Haematoxylum campechianum* L.). That is why BioGnost developed a synthetic hematoxylin substitute which is a standardized nuclear staining technique, has a longer working life than natural hematoxylin, and can be used in automated tissue stainers. ErioGnost is a reliable hematoxylin substitute for several routine and special stains. It can be used for monochromatic selective nuclear staining or one-step dichromatic staining that could replace metal-hemateins and H&E. The main advantages of ErioGnost include its stability and the reliable supply of the parent dye.



Human skin stained with ErioGnost.



Human kidney stained with ErioGnost.



Human tonsil stained with ErioGnost.

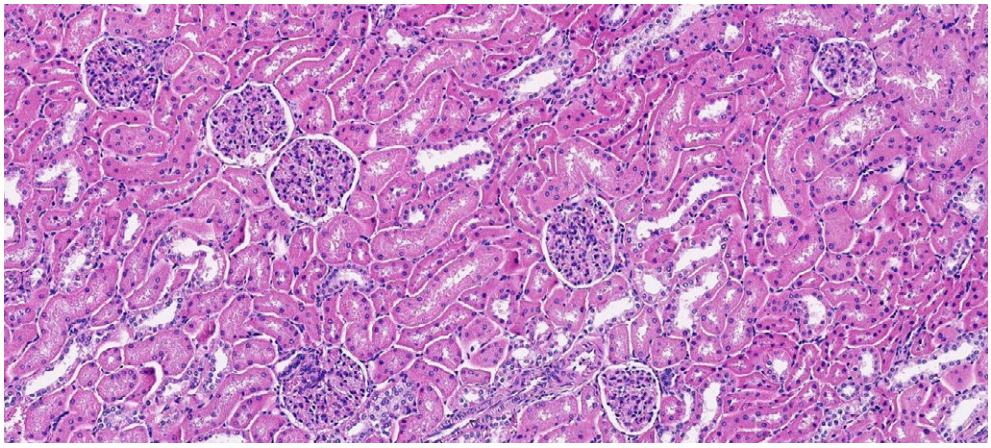
NEW

Check our staining results!

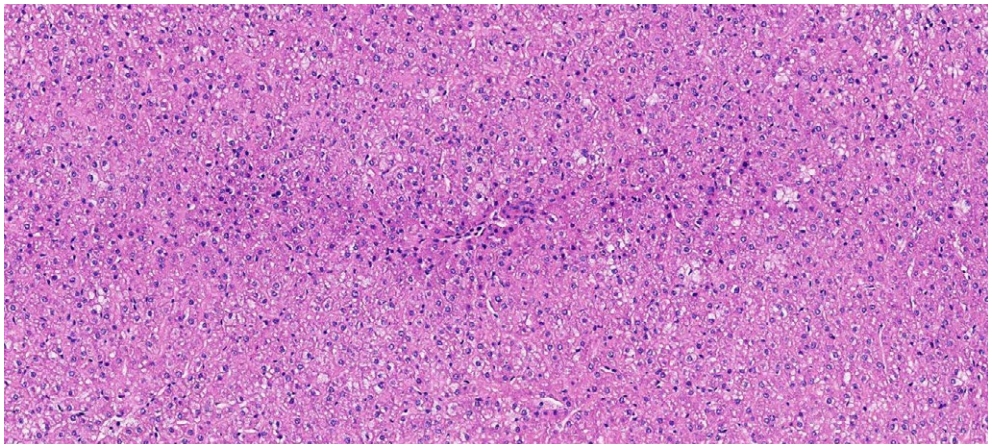
Formalin substitute

BioFix GL

BioFix GL, a formalin-free fixative solution, is an innovative reagent that allows optimal tissue fixation at the structural and molecular level, combined with the absence of toxicity and carcinogenic activity. It is excellent for conventional histology, special stains and immunohistochemistry. The main component of BioFix GL is glyoxal, the major substitute for neutral-buffered formalin in histopathologic tissue examination. It has a unique structure that allows for rapid penetration of the tissue, non-cross linking of proteins and provides an increased safety profile in comparison with formalin. Glyoxal is a larger molecule than formaldehyde. It penetrates cells/tissue quickly, fixing the proteins, and preserving the cellular morphology similar to formaldehyde. Routine H&E preparations exhibit clarity and cellular detail. All tissues fixed in BioFix GL can be stained with BioGnost H&E staining reagents with optimized protocols.



Sheep kidney stained with H&E, fixative: BioFix GL.



Sheep liver stained with H&E, fixative: BioFix GL.

Why is H&E staining so important?

Most of pathologists' everyday work is based on H&E staining results. It is extremely important to have good staining protocols because H&E staining is the basis of every histopathology laboratory. Lots of different stains have been developed, but neither of them can replace H&E. The original H&E method was developed almost 150 years ago and it still remains unchanged. However, there is estimation that 50% of worldwide histopathology laboratories have serious problems with the quality of H&E staining and consequently with making a reliable diagnosis. H&E staining results of good quality are very rare because of lack of knowledge in histotechnology. That is why BioGnost is here to help you and offer the right solution for your everyday problem.

H&E stains for every preference

BioGnost's H&E stain optimization program

BioGnost's expert team is here for you. We are available at any time to help you choose the right stain for you, optimize the protocol that meets your needs and give your histotechnicians and pathologists the quality and performance they expect. With our knowledge and experience we can help you to improve your staining protocol and workflow. Just send us your slides and choose the desired H&E combinations to test. Your slides will be stained with BioGnost's staining solutions according to your lab needs. We will scan the slides for you so you will quickly get the results you want.

Contact	Send	Optimize	Scan & choose
■ tell us your H&E staining preferences or choose combinations you would like to be tested ■ tell us your problems ■ we will send you a questionare	■ send us your slides of tissue samples that you wish to be tested and optimized (2 stained and 5 unstained slides) ■ send us your exact protocol and type of stainer you use	■ our team of experts will optimize the protocol for you with our staining reagents according to your preferences	■ we will send you scans with detailed protocols and reagents used so you can choose what suits your lab the most

We prefer to maintain regular contact with your laboratory even after the optimization process is done so feel free to reach to us for any further questions. BioGnost's team of experts is here to consult you and lead you to better staining results and accurate diagnosis. Our experience in clinical laboratory settings, stain product development, and troubleshooting, ensure appropriate recommendations for your laboratory. Don't waste your time trying to find the best protocol, just contact us and we will help you!

References

1. Bancroft JD, Gamble M: Theory and Practice of Histological Techniques. 6th Ed., Churchill-Livingstone Elsevier, Nottingham, UK, 2008.
2. Carson FL, Hladik C: Histotechnology A Self-Instructional Text. 3rd Ed., ASCP Press, Dallas, TX, 2009.
3. Guidelines for hematoxylin & eosin staining: National Society for Histotechnology
4. Kiernan JA, Horobin RW. A special issue devoted to hematoxylin, hematein, and hemalum. Biotech Histochem. 2010;85:5-6. doi: 10.3109/10520290903048368.
5. Stefanović D, Stefanović M, Lalošević D. Use of eriochrome cyanine R in routine histology and histopathology: is it time to say goodbye to hematoxylin? Biotech Histochem. 2015;90:461-469. doi: 10.3109/10520295.2015.1057765.
6. DeJarnatt V, Criswell SL. Glyoxal: a proposed substitute for formalin in H&E and special stains. J Histotechnol. 2021;44:37-45. doi: 10.1080/01478885.2020.1830664.

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