PathnSitu Biotechnologies

Laboratory Use Only CE

Verhoeff -Vangieson (VVG) Stain

PRODUCT INFORMATION:
SSP021 25 Reactions Ready to use
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PERFORMANCE CHARACTERISTICS:
Staining Interpretation:
Elastic Fibers: Black
Cell Nuclei: Blue-black
Collagen: Red
Other tissue elements: Yellow

SUMMARY AND EXPLANATION

For *laboratory* use only

VVG Stain Kit is intended for use in histological demonstration of Elastic fibres i.e to identify the presence or absence of elastic fibres in tissues. These fine elastic fibers cannot typically be seen on routine haematoxylin and eosin (H&E)-stained tissue sections and they may appear refractile on H and E staining, but often cannot be clearly distinguished from collagen fibers and smooth muscle. Therefore, the Verhoeff stain enables visualization of these fine structures under traditional light microscopy. This stain is useful in demonstrating atrophy of elastic tissue in cases of emphysema, and the thinning and loss of elastic fibers in arteriosclerosis, and other vascular diseases.

PRINCIPLE OF THE PROCEDURE

In the Verhoeff-Van Gieson (VVG) staining method, the tissue is stained with hematoxylin-ferric chloride and iodine containing working solution.
Elastic fibres have the strongest affinity for the hematoxylin-ferric chloride iodine solution which is used to overstain the entire tissue section. Therefore, the elastic fibres retain the dye, even after a diluted solution of ferric chloride is used for differentiation to break the tissue-mordant-dye complex in the other tissue elements. Sodium thiosulfate is used to remove excess iodine. The subsequent Van Gieson counter stain utilizes picric acid and acid fuchsin to stain collagen and muscle fibres, producing contrast against the hematoxylin stain. Under-differentiation is preferred in the Verhoeff stain, as picric acid used in the van Gieson counter stain serves to differentiate the elastic fibres further.

Kit Contents	Product Code	Storage Conditions	Pack Sizes	
			25 tests	50 tests
Hematoxylin Solution,5% (Reagent A)	IPS088	RT	15ml	25ml
Ferric Chloride Solution,10% (Reagent B)	IPS089	RT	7ml	15ml
Lugol's Iodine Solution (Reagent C)	IPS090	RT	7ml	15ml
Ferric Chloride, 2% (Reagent D)	IPS091	RT	25ml	50ml
Sodium Thiosulphate Solution, 5% (Reagent E)	IPS051	RT	25ml	50ml
Vangieson Counter Stain (Reagent F)	IPS092	RT	25ml	50ml

STORAGE AND HANDLING

Storage Recommendations: Store at Room Temperature. When stored at the appropriate conditions, the reagents are stable until expiry. **Do not use the reagents after expiration date provided on the vial.**
To ensure proper regent delivery and stability, replace the dispenser cap after every use and immediately place the vials at room temperature away from sunlight in an upright position.

SPECIMEN PREPARATION

RECOMMENDED POSITIVE CONTROLS:
Formalin-fixed Paraffin-Embedded Human Lung, Skin, Artery or any vascular tissue. Cut the sections, usually 4-5microns thick

PRECAUTIONS

1. Normal precautions exercised in handling laboratory reagents should be followed.
2. This product should be used by qualified and trained professional users only
3. The product contains Alcohol and is classified as highly-flammable, must be kept away from ignition sources
4. It can cause serious eye and skin irritation. Refer to Material Safety Datasheet for any updated risk, hazard or safety information.
5. Dispose of waste observing all local, state, provincial or national regulations.
6. Do not use reagents after expiration date
7. Use protective clothing and gloves, while handling reagents
8. Avoid microbial contamination of reagents as it may lead to incorrect results

MATERIALS REQUIRED,BUT NOT PROVIDED:

- Xylenes
- Graded alcohols (50%, 70%, 95%, absolute)
- DPX Mountant
- Microscopic slides (positively charged)
- Slide holder
- Cover slips
- Coplin jars

PREPARATION OF WORKING SOLUTION

Verhoeff-Vangieson Working Solution: Verhoeff-vangieson working solution should be made up fresh for the best results. Prepare the working solution by adding the following reagents in the similar order:

- 5% Hematoxylin solution (Reagent A): 0.5 ml
- 10% Ferric chloride (Reagent B) : 0.25 ml
- Lugol's Iodine solution (Reagent C) : 0.25 ml

The above mentioned volumes are good enough to use for 1 slide. Mix the above amounts (or needed proportions thereof) well. Solution should be jet black. Use immediately and any leftover volume can be added to the slides during the incubation of this working solution.

STAINING PROCEDURE:

1. Deparaffinize and hydrate slides to distilled water. Stain in verhoeff-vangieson working solution for 60 minutes. Tissue should be completely black.
2. Rinse in tap water with 2-3 changes and rinse in distilled water with 2-3 changes.
3. Differentiate in 2% ferric chloride solution (Reagent D) for 5-10 seconds with mild agitation.
   Note: As the time of differentiation is somewhat dependent on the amount of elastic tissue present, it is better not to rely on the control of timing for the differentiation of all sections, and slides must be individually differentiated to get good results, and also, it is better to rely on the side of under differentiation.
4. Stop differentiation with several changes of tap water and check microscopically for black elastic fiber staining and gray background.
   (It is better to slightly under differentiate the tissue, since the

US Office:1257, Quarry Ln, Suite150, Pleasanton, CA-94566, USA Ph: +1 925-218-6939,
India Office:PS Heights, Plot# A-28/1/11B/B, Road#, 15, Industrial Development Area, Nacharam, Secunderabad-76
Phone: 040-27015544/33,Fax:040-2701 5544 , **E-Mail:** customerservice@pathnsitu.com **Web:** www.pathnsitu.com

subsequent Van Gieson's counter stain can extract the elastic stain)

5. Wash slides in tap water.
6. Treat with 5% sodium thiosulfate solution (Reagent E) for 1 minute. Discard solution. Wash in running tap water for 3-5 minutes.
7. Counter stain in Van Gieson's solution (Reagent F) for 2-3 minutes. (Counterstaining with Van Gieson's solution should not be prolonged, as picric acid present in this step differentiates the sections further)
8. Dehydrate quickly through 95% alcohol, 2 changes of 100% alcohol.
9. Clear in 3 changes of xylene for 2 minutes each.
10. Cover slip with Compatible mounting medium (DPX).

PERFORMANCE CHARACTERISTICS

Elastic Fibres	**:** Black
Cell Nuclei	**:** Blue-black
Collagen	**:** Red
Other tissue elements	**:** Yellow

TROUBLESHOOTING

1. Follow the specific protocol recommendations according to data sheet provided
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining. Improper fixation, tissue processing, freezing, thawing, washing, drying, heating, sectioning or contamination with other tissues or fluids may produce artifacts, reagent trapping or inaccurate results
3. Do not allow the section to dry out during the entire staining process
4. Excessive or incomplete counterstaining may compromise the interpretation of the results
5. If unusual results occur, contact PathnSitu Technical Support at +91-40-2701 5544 or E-mail: techsupport@pathnsitu.com

LIMITATIONS AND WARRANTY

Authorized and skilled personnel only may use the product. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic test results. A qualified pathologist must perform the evaluation of the test results. There are no warranties, expressed or implied, which extend beyond the description. PathnSitu is not liable for property damage, personal injury, time or effort on economic loss caused by this product.

BIBLIOGRAPHY

1. *The utility of elastic Verhoeff-Van Gieson staining in dermatopathology*; Viktoryia Kazlouskaya 1, Saurabh Malhotra, Jennifer Lambe, Munir Hassen Idriss, Dirk Elston, Christian Andres
2. *A histological study on the distribution of dermal collagen and elastic fibres in different regions of the body*; Naveen Kumar1, Pramod Kumar2, Keerthana Prasad and B. Satheesha Nayak1
3. *A modified Verhoeff-van Gieson elastin histochemical stain to enable pulmonary arterial hypertension model characterization* K.R. Percival, Z.A. Radi Pfizer Worldwide Research and Development, Drug Safety R&D, Andover, MA, USA

EXPLANATION OF SYMBOLS

LOT- Lot number / Batch number

- Expiry

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RT- Room Temperature

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