epredia Enhancing precision cancer diagnostics

Signature Series™ Stains
Instructions for Use

Intended Use

The Signature Series™ Staining Products are in vitro diagnostic medical devices intended to be used by laboratory professionals for the qualitative evaluation of tissue morphology in pathology specimens.

Hematoxylin is intended be used as a nuclear stain for the diagnosis of general pathology specimens.

Eosin is intended to be used as a cytoplasmic stain for the diagnosis of general pathology specimens.

Clarifier™ is intended to be used as an aid in hematoxylin staining for the diagnosis of general pathology specimens.

Bluing Reagent is intended to be used as an aid in hematoxylin staining for the diagnosis of general pathology specimens.

Introduction

Epredia™ Signature Series™ stains and ancillary reagents have been designed as a family of histology staining products that include hematoxylin, Eosin-Y, Bluing Reagent, and Clarifier™. All products are intended to be used together as a group.
Signature Series stains are formulated and packaged to provide our customers with the highest quality and consistency in the histology market. The hematoxylin products do not contain mercury-based oxidizing agents. The positively charged aluminum-hematein dye lake complex combines with the negatively charged phosphate groups of nuclear chromatin, forming a distinct blue-purple color characteristic of hematoxylin stains.

Each lot of stain is tested by perhaps the most stringent quality assurance in the industry. Quality assurance testing consists of testing at the raw material, work in process, and finished good stages. As a final check, each lot is used to stain tissue compared against previous lots to guarantee consistent and reliable results.

Signature Series Stains are available in convenient pint and gallon bottles. All stains are ready to use and require no dilution. All dyes are certified by the Biological Stain Commission.

Hematoxylin 7211 & 7212

Hematoxylin 7211 is a rapid, progressive hematoxylin that has gained a wide market share and recognition in the histology and cytology fields. The product is a hybrid between classical Harris and Gill formulations: it provides the classic intensity and hues of a Harris hematoxylin and the rapid progressive qualities of a Gill hematoxylin. Differentiation is not necessary, but Clarifier 1 should be used. Clarifier 1 will eliminate any background staining that may be caused by the use of excess gelatin or other adhesives in the water bath. Clarifier 2 can be used as well; however, a shorter immersion time is required. Hematoxylin 7211 does not have an affinity for acid mucopolysaccharide (mucin) staining, as do some other hematoxylin formulations. It provides consistent, well-delineated nuclear staining results and does not require daily filtering.

Hematoxylin 7212 is a darker and more color intensive version of Hematoxylin 7211. It contains most of the attributes and features of the Hematoxylin 7211 stain; however, Hematoxylin 7212 does have a slight affinity for mucin staining. Both Hematoxylin 7211 and 7212 can be used for immunohistochemistry and special staining techniques.

Hematoxylin 7211 has a distinct feature: it forms crystals during the cold winter months. This feature generally occurs during shipping or if the hematoxylin is stored in a cold environment. Crystal formation is due to the mordant being put into solution at its saturation point. These crystals have no adverse effect on staining and may be allowed to remain in the bottle. However, if desired, the stain can be adjusted in one of two ways:

- If there is a small amount of crystals in the bottle: Filter the hematoxylin prior to use; once filtered, the hematoxylin will remain crystal-free at room temperature.
- If there is an abundance of crystals in the bottle: Gently warm the hematoxylin with constant stirring on a stir plate until the crystals go back into solution and then filter. DO NOT BOIL!

Hematoxylin 7212 will not form an abundance of crystals due to utilizing a different mordant. If a small amount of crystals is present, they may be allowed to remain in the bottle or they can be filtered as described above.

Hematoxylin 1 & Hematoxylin 2

Hematoxylin 1 is a rapid, progressive hematoxylin designed to produce staining characteristics, intensities, and hues similar to Gill 1 & 2 hematoxylins. Differentiation is not necessary, but Clarifier 1 should be used. Clarifier 1 will eliminate background staining that may be caused by the use of excess gelatin or other adhesives in the water bath. Clarifier 2 can be used as well; however, a shorter immersion time is required.

Hematoxylin 2 is a darker and more color intensive version of the Hematoxylin 1 stain. Differentiation is not necessary, but Clarifier 2 should be used. Clarifier 2 will eliminate background staining that may be caused by the use of excess gelatin or other adhesives in the water bath.

Hematoxylin 1 and 2 can be used for immunohistochemistry and special staining techniques. Hematoxylin 2 is recommended for frozen section staining. Both Hematoxylin 1 and 2 are ready to use, do not require filtration prior to use, and do not form crystals upon exposure to cold environments.

Clarifier 1

Clarifier 1 is a reagent designed to eliminate background staining sometimes caused by excessive adhesives in the water bath such as gelatin. Clarifier 1 selectively removes hematoxylin staining from excess adhesive without affecting nuclear staining. This product is only effective with progressive staining and should not be used with regressive stains.

Clarifier 1 is designed for use after staining with progressive hematoxylins. After tissues have been sufficiently stained with a progressive hematoxylin, slides are removed from the stain and excess hematoxylin is removed by rinsing the sections in water. The sections are then placed into Clarifier 1 for 30 seconds to 1 minute. A 1-minute water rinse should follow as this stops the reaction and rinses excess acid from the tissue section. If Clarifier 1 is not successful in removing the background staining, use Clarifier 2 in its place with a possible time reduction.

Clarifier 2

Clarifier 2 is a reagent designed to eliminate background staining sometimes caused by excessive adhesives in the water bath such as gelatin. Clarifier 2 selectively removes hematoxylin staining from excess adhesive without affecting nuclear staining. This product is only effective with progressive staining and should not be used with regressive stains. Clarifier 2 was designed to be used in histology only.

Clarifier 2 is designed for use after staining with progressive hematoxylins. After tissues have been sufficiently stained with a progressive hematoxylin, slides are removed from the stain and excess hematoxylin is removed by rinsing the sections in water. The sections are then placed into Clarifier 2 for 20 seconds to 40 seconds. A 1-minute water rinse should follow as this stops the reaction and rinses excess acid from the tissue section.

Bluing Reagent

Bluing Reagent is a buffered alkaline rinse that shifts the final hue of hematoxylin from reddish-blue to traditional blue-purple. Unlike ammonia water and lithium carbonate, Bluing Reagent inhibits pH changes that can adversely affect nuclear detail and "crispness".

Conventional bluing reagents often require lengthy water wash rinses after use; however, Bluing Reagent requires only a 1-minute wash in water. Additionally, unlike ammonia-based solutions, Bluing Reagent will not cause lifting or loss of sections from slides.

Eosin-Y

Eosin-Y is an acidified, alcoholic counterstain that provides excellent delineation of cytoplasmic components and stains the cytoplasm of muscle, red blood cells, and connective tissue three distinct color hues of pink to red. Eosin-Y provides excellent contrast between cytoplasmic components and the bluish-purple hues of the nuclear chromatin.

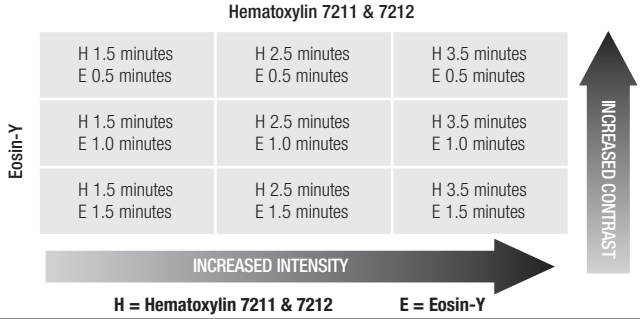
When used as a cytoplasmic stain in the hematoxylin and eosin staining procedure, a 70% or 95% alcohol rinse should precede the Eosin-Y, and three anhydrous dehydrating reagents should follow the Eosin-Y. These steps will minimize pH changes in the eosin and will help to prevent water carryover, respectively. If desired, a 95% alcohol step may be inserted after the Eosin-Y to help lighten the overall intensity of cytoplasmic staining.

Hematoxylin 7211 and 7212 Recommended Staining Procedure

Station	Solution	Time
1	Clear-Rite™ 3 or Xylene	3 minutes
2	Clear-Rite 3 or Xylene	3 minutes
3	Clear-Rite 3 or Xylene	3 minutes
4	100% Flex™ or Reagent Alcohol	1 minute
5	100% Flex or Reagent Alcohol	1 minute
6	100% Flex or Reagent Alcohol	1 minute
7	95% Flex or Reagent Alcohol	1 minute
8	Rinse in running tap water	Briefly
9	Deionized or distilled water	Rinse
10	Hematoxylin 7211 or 7212	Select time from Grid
11	Deionized or distilled water	Rinse off excess stain
12	Clarifier 1 or 2	20 seconds to 1 minute
13	Deionized or distilled water	1 minute (agitate)
14	Bluing Reagent	1 minute
15	Deionized or distilled water	1 minute
16	95% Flex or Reagent Alcohol	Rinse
17	Eosin-Y	Select time from Grid
18	100% Flex or Reagent Alcohol	1 minute
19	100% Flex or Reagent Alcohol	1 minute
20	100% Flex or Reagent Alcohol	1 minute
21	Clear-Rite 3 or Xylene	1 minute
22	Clear-Rite 3 or Xylene	1 minute
23	Clear-Rite 3 or Xylene	1 minute

Note: This procedure may not fit every situation. Modifications may be necessary.

Hematoxylin 7211 and 7212 Staining Grid



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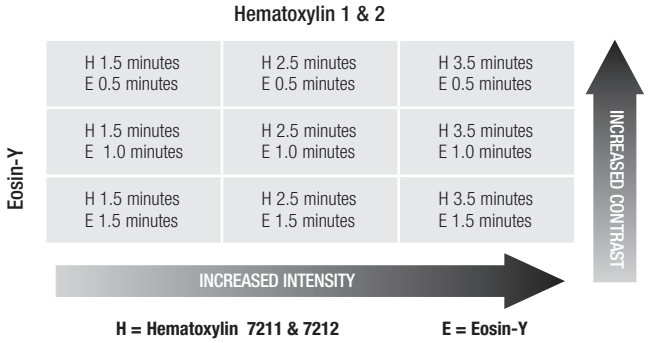
Hematoxylin 1 and 2 Recommended Staining Procedure

Station	Solution	Time
1	Clear-Rite 3 or Xylene	3 minutes
2	Clear-Rite 3 or Xylene	3 minutes
3	Clear-Rite 3 or Xylene	3 minutes
4	100% Flex or Reagent Alcohol	1 minute
5	100% Flex or Reagent Alcohol	1 minute
6	100% Flex or Reagent Alcohol	1 minute
7	95% Flex or Reagent Alcohol	1 minute
8	Rinse in running tap water	Briefly
9	Deionized or distilled water	Rinse
10	Hematoxylin 1 or 2	Select time from Grid
11	Deionized or distilled water	Rinse off excess stain
12	Clarifier 1 or 2	20 seconds to 1 minute
13	Deionized or distilled water	1 minute (agitate)
14	Bluing Reagent	1 minute
15	Deionized or distilled water	1 minute
16	95% Flex or Reagent Alcohol	Rinse
17	Eosin-Y	Select time from Grid
18	100% Flex or Reagent Alcohol	1 minute
19	100% Flex or Reagent Alcohol	1 minute
20	100% Flex or Reagent Alcohol	1 minute
21	Clear-Rite 3 or Xylene	1 minute
22	Clear-Rite 3 or Xylene	1 minute
23	Clear-Rite 3 or Xylene	1 minute

Note: This procedure may not fit every situation. Modifications may be necessary.

Hematoxylin 1 and 2 Staining Grid



Frozen Sections Using Hematoxylin 2 Staining Procedure

Optional: Fix sections in cold Pen-Fix to which 1 mL of glacial acetic acid has been added. Rinse in deionized or distilled water for 4-5 dips. Note: Sections fixed in this solution will demonstrate brighter nuclear staining.

Station	Solution	Time
1	Hematoxylin 2	3-4 dips
2	Deionized or distlled water	5-10 dips
3	Bluing Reagent	3-4 dips
4	Deionized or distilled water	3-4 dips
5	95% Flex or Reagent Alcohol	3-4 dips
6	Eosin-Y	1-2 dips
7	100% Flex or Reagent Alcohol	1-2 dips
8	100% Flex or Reagent Alcohol	5-10 dips
9	100% Flex or Reagent Alcohol	5-10 dips
10	Xylene	10 dips
11	Xylene	10 dips
12	Xylene	10 dips

Note: This procedure may not fit every situation. Modifications may be necessary.

The laboratory should develop a product rotation and change out schedule that adheres to the policies of their facility.

Warnings and Precautions

See Safety Data Sheets for warnings and precautions, as well as EUH code definitions.
See container label for warnings and precautions.

Order Information

Product	Size	Qty.	REF
Hematoxylin 7211	1 pt. (0.47 L)	4/cs.	7211
Hematoxylin 7212	1 pt. (0.47 L)	4/cs.	7212
Hematoxylin 1	1 pt. (0.47 L)	4/cs.	7221
Hematoxylin 2	1pt. (0.47 L)	4/cs.	7231
Clarifier™ 1	1 gal. (3.79 L)	Ea.	7401
Clarifier™ 1	1 gal. (3.79 L)	4/cs.	7441
Clarifier™ 2	1 gal. (3.79 L)	Ea.	7402
Clarifier™ 2	1 gal. (3.79 L)	4/cs.	7442
Bluing Reagent	1 gal. (3.79 L)	Ea.	7301
Bluing Reagent	1 gal. (3.79 L)	4/cs.	7341
Eosin-Y	1 pt. (0.47 L)	4/cs.	7111
Hematoxylin 7211	5 L (1.32 gal)	Ea.	7211L
Hematoxylin 7212	5 L (1.32 gal)	Ea.	7212L
Hematoxylin 1	5 L (1.32 gal)	Ea.	7221L
Hematoxylin 2	5 L (1.32 gal)	Ea.	7231L
Clarifier™ 2	5 L (1.32 gal)	Ea.	7402L
Bluing Reagent	5 L (1.32 gal)	Ea.	7301L
Eosin-Y	5 L (1.32 gal)	Ea.	7111L

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