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Thermo Scientific Richard-Allan Scientific Histology Signature Series Stains Instructions for Use

For in vitro diagnostic use.
For use in histological and cytological staining procedures.

Thermo Scientific™ Richard-Allan Scientific™ Signature Series™ Stains have been designed as a family of histology staining products that include Thermo Scientific™ Hematoxylin, Eosin-Y, Bluing Reagent, and Clarifier. All stains are intended to be used together as a family of products.

All Thermo Scientific 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 Thermo Scientific stains 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 one pint bottles or gallon containers. All stains are ready to use and require no dilutions. All dyes are certified by the Biological Stain Commission.

Hematoxylin 7211 & 7212

Thermo Scientific™ Hematoxylin 7211 is a rapid progressive hematoxylin that has gained a wide market share and notoriety 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 formulation and the rapid and progressive qualities of a Gill Hematoxylin formulation. 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 will also provide a consistent, well delineated nuclear stain and does not require daily filtering.

Hematoxylin 7212 is a darker and more color intensive version of the Hematoxylin 7211 stain. It contains most of the attributes and features of the Hematoxylin 7211 stain. 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. This is due to the mordant being put into solution at its saturation point. The crystals are sometimes viewed as a hindrance in the market place because it is thought that the potency of the Hematoxylin subsides as more slides are stained. Actually, the crystals have no adverse effects on the staining quality of the Hematoxylin and the stain can be adjusted in one of two ways:

- If 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 filter. DO NOT BOIL! Hematoxylin 7212 will not form an abundance of crystals. It contains a unique metal mordant that is stable. If a small amount of crystals are present, follow Step 1 listed above.

Hematoxylin 1 & Hematoxylin 2

Thermo Scientific™ Hematoxylin 1 is a progressive stain developed to be used as a nuclear stain in the Hematoxylin and Eosin-Y staining procedure and in cytology. The stain requires no filtration prior to use and will not form crystals upon standing or exposure to cold weather.

Hematoxylin 1 was designed to produce staining intensities similar to Gill Hematoxylins 1 & 2. Hematoxylin 1 will give different nuclear staining intensities based on the time that tissue sections are allowed to remain in the solution. 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 2 is a darker and more color intensive version of the Hematoxylin 1 stain. It contains most of the attributes and features of Hematoxylin 1. Differentiation is not necessary, but Clarifier 2 should be used. Clarifier 2 will eliminate any background staining that may be caused by the use of excess gelatin or other adhesives in the water bath. Dipping slides in a dilute acid alcohol (HCl) can also be used, but is not recommended.

Hematoxylin 1 and 2 can be used for immunohistochemistry and special staining techniques. Hematoxylin 2 is recommended for frozen section staining.

Clarifier 1

Thermo Scientific™ Clarifier 1 is a reagent designed to eliminate background staining 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 designated 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 in 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

Thermo Scientific™ Clarifier 2 is a reagent designed to eliminate background staining 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 designated 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 in 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

Thermo Scientific™ Bluing Reagent is used to enhance the color of the nuclei after hematoxylin staining. This is achieved by changing the nuclear chromatin stain from a reddish-blue to a crisp blue-purple. This reaction is pH dependent and is most likely the result of chelation.

Bluing Reagent is a buffered product that ensures a proper alkalinity (pH = 8.0). Unlike ammonia water and lithium carbonate, Bluing Reagent does not allow for the pH shift that can affect the crispness of nuclear detail. Conventional bluing reagents require a 5 minute water wash after use. Bluing Reagent requires only a 1 minute wash in water. Bluing Reagent will not allow sections to be removed from the slides.

Eosin-Y

Thermo Scientific™ Eosin-Y is an acidified, alcoholic counterstain that provides excellent delineation of cytoplasmic components in shades of red and pink. Muscle, red blood cells and connective tissue all stain various shades of red and pink. Eosin-Y provides excellent contrast between the cytoplasmic components and nuclear chromatin and will accentuate the bluish purple hues of the nuclear chromatin.

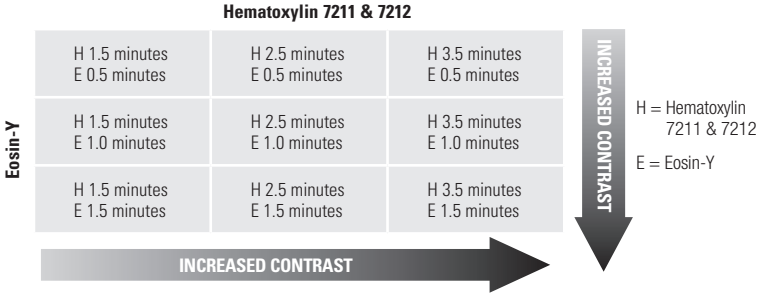
When used as a cytoplasmic stain in the hematoxylin and eosin staining procedure, a 70% or 95% alcohol rinse should precede the Eosin-Y, and three anhydrous dehydrating reagents should follow the Eosin-Y. This will alleviate any water carry over or changes in the pH of the Eosin-Y solution. A 95% alcohol rinse can be used after the Eosin-Y to help lighten the intensity of the cytoplasmic stain.

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	Running tap water	Rinse off excess stain
12	Clarifier 1 or 2	30 seconds to 1 minute
13	Rinse in running tap water	30 seconds (agitate)
14	Bluing Reagent	1 minute
15	Rinse in running tap 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

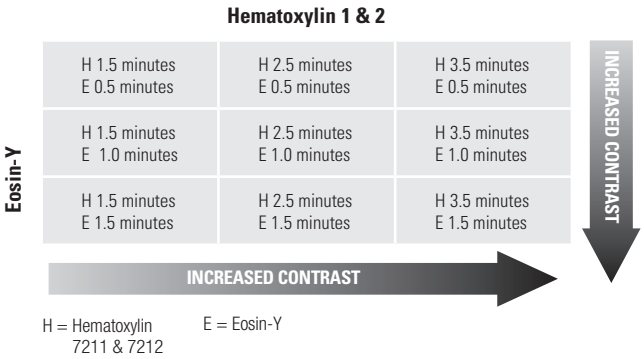


Hematoxylin 1 and 2 Recommended Staining Procedure

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	Running tap water	Rinse off excess stain
12	Clarifier 1 or 2	20 seconds to 1 minute
13	Rinse in running tap water	30 seconds (agitate)
14	Bluing Reagent	1 minute
15	Rinse in running tap 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: Tissue sections fixed in the following solution produces a brighter nuclear stain:
Fix sections in cold Pen-Fix that has added to it 1 mL glacial acetic acid. Rince in tap water for 4-5 dips.

Station	Solution	Time
1	Hematoxylin 2	3-4 dips
2	Rinse off excess stain	5-10 dips
3	Bluing Reagent	3-4 dips
4	Rinse in tap water	3-4 dips
5	95% Flex or Reagent Alcohol	3-4 dips
6	Eosin-Y	1-2 dips
7	Rinse in tap water	1-2 dips
8	100% Flex or Reagent Alcohol	1-2 dips
9	100% Flex or Reagent Alcohol	5-10 dips
10	100% Flex or Reagent Alcohol	5-10 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 department.

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

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Anatomical Pathology

15 C (59 F) – 30 C (86 F)

IS8122 R12/15

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