

Thermo Scientific Richard-Allan Scientific Histology and Cytology Standard Stains Instructions for Use

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

The Thermo Scientific™ Richard-Allan Scientific™ family of histology and cytology staining products are a family designed to include multiple strength Hematoxylin, variations of Eosin-Y, Bluing Reagent, Cytology Counterstains, and Differentiating Solution. All stains are intended to be used together as a family of products. All our stains are formulated and packaged to provide our customers with the highest quality and consistency in the histology and cytology markets. 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. Cytology counterstains are offered in a variety of strengths and provide the end user with control of the cytoplasm color intensities and hues.

Each lot of Thermo Scientific 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.

Thermo Scientific Standard Stains are available in convenient one pint bottles or gallon containers. All dyes used in these formulations are certified by the Biological Stain Commission.

Gill Hematoxylin 1, 2 and 3

Thermo Scientific™ Gill Hematoxylin 1, 2, and 3 are rapid progressive hematoxylin stains that are widely known and respected in the Histology and Cytology fields. They offer the convenience and choice of stain intensity. The Thermo Scientific™ Gill Hematoxylin series is designed to control over-oxidation. Thermo Scientific™ Differentiation is usually not necessary, however Thermo Scientific™ Clarifier 1 or a dilute glacial acetic acid rinse should be used with this series of stains. Thermo Scientific Clarifier 1 will eliminate any background staining that may be caused by the use of excess gelatin or other adhesives in the water bath. Thermo Scientific™ Clarifier 2 can be used as well, however a shorter immersion time is required. These stains will provide a familiar, consistent, well delineated nuclear stain, are ready to use and do not require dilution. Stain filtering may be needed to remove sheen caused by oxidation or cells shed off of slides in the cytology staining process. All three Gill stains are stable and will not precipitate in colder environments.

Gill 1 (2.0 g/L) is the least intense stain and is most commonly used in cytology and in special stains.

Gill 2 (4.0 g/L) is a moderately intense stain and is most commonly used in histology, with some limited use in cytology.

Gill 3 (6.0 g/L) is an intense stain and is exclusively used in histology and also for the staining of plastic sections.

Modified Harris Hematoxylin

Thermo Scientific™ Modified Harris Hematoxylin is an acidified formulation that provides traditional nuclear staining results. Harris Hematoxylin procedures require acid alcohol differentiation. The nuclear chromatin and cytoplasm are over stained and the acid alcohol is used to destain the cytoplasm and also remove excess staining from the nuclear chromatin. Staining times are typically in the 5 minute range. Our Differentiating Solution can be used successfully with Modified Harris Hematoxylin.

Our Thermo Scientific Modified Harris Hematoxylin is more stable than most commercial Harris Hematoxylin. It will not abundantly auto-oxidize at the same accelerated rate as other Harris Hematoxylin. The potency of the stain will slowly change, however the staining viability will remain for longer times. The product will crystallize when exposed to colder temperatures. Simply filter the crystals or put the contents of the bottle and crystals on a heated stir plate (35° C) and the crystals will go back into solution. DO NOT BOIL! It is highly recommended to filter Modified Harris Hematoxylin on a daily basis.

Modified Mayer's Hematoxylin

Thermo Scientific™ Modified Mayer's Hematoxylin is a progressive stain that can be used with or without a glacial acetic acid rinse. The stain will provide very well defined and delineated nuclear chromatin. Due to lower dye-lake concentrations, Modified Mayer's Hematoxylin will need elevated staining time, such as 10-15 minutes to achieve darker intensities. This formulation does not contain chloral hydrate, a controlled substance.

Modified Mayer's Hematoxylin is an excellent counterstain for special stains such as the PAS stain. It is also popular and highly used as a counterstain for IHC procedures. The low dye concentration of this stain will provide excellent contrast and no masking of the primary reaction in these procedures.

Differentiating Solution

Thermo Scientific™ Differentiating Solution is an acid alcohol solution to be used with the Thermo Scientific Modified Harris Hematoxylin. It is formulated to provide as consistent results as possible for the end user. Thermo Scientific Differentiating solution is a standard 1% hydrochloric acid in 70% alcohol solution.

The mode of action of a differentiating solution is that it aggressively attacks the tissue dye-lake complex linkage in areas of excess stain in the nuclear chromatin. Also, because of weak bonding in the cell cytoplasm, the stain is easily and completely removed from these areas during differentiation. Generally two to four quick dips will be adequate for proper differentiation in Thermo Scientific Differentiating Solution. A 30-45 second water rinse following the differentiation is recommended to stop the reaction and remove excess acid from the tissue.

Eosin-Y Alcoholic

Thermo Scientific™ Eosin-Y Alcoholic is an alcoholic, acidified counterstain. This product will stain cytoplasmic components three distinct color hues. Muscle, red blood cells and connective tissue all stain various shades of red and pink.

Thermo Scientific Eosin-Y Alcoholic will provide a clear contrast between the cytoplasm and nuclei of the cell. No dilution is necessary for this product as it is ready for use. The pH of this Eosin product is between 4.8 and 5.2. Typical staining times are 30-90 seconds.

Eosin-Y w/ Phloxine

Thermo Scientific™ Eosin-Y w/ Phloxine is an alcoholic, acidified counterstain. Phloxine B has been added to brighten the cytoplasmic stain and give a more intense red color to the connective tissue, especially muscle. Inclusion bodies are better demonstrated by the inclusion of phloxine-B. Some pathologists prefer the look and range of cytoplasmic colors it provides. No dilution is necessary and it is ready for use.

Eosin-Y Saturated

Thermo Scientific™ Eosin-Y Saturated is an alcohol based, rapid cytoplasmic stain. It does not contain glacial acetic acid, so its pH is much higher (neutral) than other eosin-y alcoholic counterstains. This stain will provide intensities similar to other commercially available eosin-y products.

Thermo Scientific Eosin-Y Saturated can be used as is or diluted for use. If the product is diluted, it is recommended to dilute with 70% alcohol to the desired strength.

Thermo Scientific Gill Hematoxylin 1, 2, & 3, Eosin-Y Alcoholic, Eosin-Y w/ Phloxine, and Eosin-Y Saturated 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	Gill Hematoxylin 1, 2 or 3	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 Alcoholic, w/Phloxine, or Saturated	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.

Gill Hematoxylin 1, 2 and 3, Eosin-Y Alcoholic, Eosin-Y w/ Phloxine, or Eosin-Y Saturated Staining Grid

Gill Hematoxylin 1, 2 and 3					
Eosin-Y Alcoholic, Eosin-Y w/Phloxine, Eosin-Y Sat.	H 1.0 minutes	H 2.0 minutes	H 3.0 minutes	INCREASED CONTRAST ↓	H = Hematoxylin E = Eosin-Y
	E 0.5 minutes	E 0.5 minutes	E 0.5 minutes		
	H 1.0 minutes	H 2.0 minutes	H 3.0 minutes		
	E 1.0 minutes	E 1.0 minutes	E 1.0 minutes		
	H 1.0 minutes	H 2.0 minutes	H 3.0 minutes		
	E 1.5 minutes	E 1.5 minutes	E 1.5 minutes		
INCREASED CONTRAST →					

EA 36, 50, 65, and Modified EA

Thermo Scientific™ EA 36, 50, 65 and Thermo Scientific™ Modified EA are cytoplasmic counterstains used for all gynecological and non-gynecological specimens. These products contain common dyes and reagent solvents used in traditional EA formulations. A well preserved or fixed slide with adequate exposure to 95% alcohol or a commercial spray fixative is highly recommended for optimal staining results. Cellular results are consistent as well with basal or intermediate cells staining a blue-green hue and surface cells staining a pink-red hue.

The multi-colored effect is due to selective dye uptake by various cells and their affinities for the specific dyes. The various formulations provide the end users with control over color intensities and hues. Stain filtering is recommended to remove cells shed off of slides in the cytology staining process.

EA 36

Thermo Scientific EA 36 is used in conjunction with Thermo Scientific™ OG 6 for the staining of gynecological specimens. It will provide a slightly lighter cytoplasmic hue than EA 50. EA 36 is recommended for end users who desire a more pastel staining result in gynecological specimens.

EA 50

Thermo Scientific EA 50 is used in conjunction with OG 6 for the staining of gynecological specimens. It will provide a very traditional staining result with common staining hues and intensities. EA 50 can also be used for non-gynecological specimens however EA 65 is recommended.

EA 65

Thermo Scientific EA 65 is used in conjunction with OG 6 for the staining of non-gynecological specimens. This formulation contains less dye resulting in lighter staining in areas of heavy mucin staining consistent with non-gynecological specimens.

Modified EA

Modified EA is used in conjunction with OG 6 or Thermo Scientific™ Modified OG for gynecological and non-gynecological specimens. This formulation is optimized to reduce precipitates that are common with other traditional EA formulations. The Thermo Scientific Modified EA staining results are most similar to Thermo Scientific EA 50 and it does not contain Bismarck Brown.

OG 6 and Modified OG

OG 6 and Modified OG are cytoplasmic counterstains that stain keratinized cells orange. A well preserved or fixed slide with adequate exposure to 95% alcohol or a commercial spray fixative is highly recommended for optimal staining results. OG 6 has a specific affinity for keratinized cells and will not stain other cellular constituents. When used in conjunction with EA stains, the end user can expect traditional staining results that are consistent and familiar. Stain filtering is recommended to remove cells shed off of slides in the cytology staining process.

OG 6

OG 6 is a traditional formulation that, when used with various EA products, will provide familiar staining results for gynecological and non-gynecological specimens.

Modified OG

Modified OG is used in conjunction with EA products for staining gynecological and non-gynecological specimens. This formulation is optimized to reduce precipitates and staining times common with traditional Thermo Scientific OG 6 formulations. Modified OG and Modified EA together will reduce filtering and staining times.

Gill 1 Hematoxylin and EA 36, 50 and 65, OG 6 Recommended Staining Procedure

Station	Solution	Time
1	95% Flex or 95% Dehydrant	3 minutes
2	95% Flex or 95% Dehydrant	2 minutes
3	Deionized or Distilled Water	30 seconds w agitation
4	Gill 1 Hematoxylin	1.0 – 1.5 minutes
5	Deionized or Distilled Water	15 seconds w/agitation
6	Clarifier 1	30 seconds
7	Deionized or Distilled Water	30 seconds
8	Bling Reagent	30 seconds
9	De-ionized or Distilled Water	30 seconds w/agitation
10	95% Flex or 95% Dehydrant	30 seconds
11	OG 6	Time chosen from grid
12	95% Flex or 95% Dehydrant	30 seconds
13	95% Flex or 95% Dehydrant	30 seconds
14	EA 36, 50, 65	Time chosen from grid
15	95% Flex or 95% Dehydrant	30 seconds
16	95% Flex or 95% Dehydrant	30 seconds
17	100% Flex or 100% Dehydrant	1 minute
18	100% Flex or 100% Dehydrant	1 minute
19	100% Flex or 100% Dehydrant	1 minute
20	Clear-Rite 3 or Xylene	1 minute
21	Clear-Rite 3 or Xylene	1 minute
22	Clear-Rite 3 or Xylene	1 minute

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

Gill 1 Hematoxylin and EA 36, 50 and 65, OG 6 Staining Grid

EA 36 50 65	OG 6			INCREASED STAINING BLUE-GREEN HUES
	OG 1.5 minutes EA 1.0 minutes	OG 2.5 minutes EA 1.0 minutes	OG 3.5 minutes EA 1.0 minutes	
	OG 1.5 minutes EA 3.0 minutes	OG 2.5 minutes EA 3.0 minutes	OG 3.5 minutes EA 3.0 minutes	
	H 1.5 minutes E 5.0 minutes	OG 2.5 minutes EA 5.0 minutes	OG 3.5 minutes EA 5.0 minutes	

INCREASED STAINING ORANGE HUES

Gill 1 Hematoxylin and Modified EA & OG Recommended Staining Procedure

Station	Solution	Time
1	95% Flex or 95% Dehydrant	3 minutes
2	95% Flex or 95% Dehydrant	2 minutes
3	Deionized or Distilled Water	30 seconds w/agitation
4	Gill 1 Hematoxylin	1.0 – 1.5 minutes
5	Deionized or Distilled Water	15 seconds w/agitation
6	Clarifier 1	30 seconds
7	Deionized or Distilled Water	30 seconds
8	Bling Reagent	30 seconds
9	Deionized or Distilled Water	30 seconds w/agitation
10	95% Flex or 95% Dehydrant	30 seconds
11	Modified OG	Time chosen from grid
12	95% Flex or 95% Dehydrant	30 seconds
13	95% Flex or 95% Dehydrant	30 seconds
14	Modified EA	Time chosen from grid

15	95% Flex or 95% Dehydrant	30 seconds
16	95% Flex or 95% Dehydrant	30 seconds
17	100% Flex or 100% Dehydrant	1 minute
18	100% Flex or 100% Dehydrant	1 minute
19	100% Flex or 100% Dehydrant	1 minute
20	Clear-Rite 3 or Xylene	1 minute
21	Clear-Rite 3 or Xylene	1 minute
22	Clear-Rite 3 or Xylene	1 minute

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

Gill 1 Hematoxylin and Modified EA and OG Staining Grid

Modified EA	Modified OG			INCREASED STAINING BLUE-GREEN HUES	OG = Modified OG EA = Modified EA
	OG 1.5 minutes EA 1.0 minutes	OG 2.5 minutes EA 1.0 minutes	OG 3.5 minutes EA 1.5 minutes		
	OG 1.5 minutes EA 3.0 minutes	OG 2.5 minutes EA 3.0 minutes	OG 3.5 minutes EA 3.0 minutes		
	OG 1.5 minutes E 5.0 minutes	OG 2.5 minutes EA 5.5 minutes	OG 3.5 minutes EA 5.0 minutes		

INCREASED STAINING ORANGE HUES

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
Eosin-Y Alcoholic	1 pt. (0.47 L) bottle	4/cs.	71204
Eosin-Y Alcoholic	1 gal. (3.79 L) bottle	Ea.	71211
Eosin-Y Alcoholic	2.5 gal. (9.46 L) cubitainer	Ea.	71225
Eosin-Y with Phloxine	1 pt. (0.47 L) bottle	4/cs.	71304
Eosin-Y with Phloxine	1 gal. (3.79 L) bottle	Ea.	71311
Eosin-Y Saturated	1 pt. (0.47 L) bottle	4/cs.	71504
Gill 1 Hematoxylin	1 pt. (0.47 L) bottle	4/cs.	72404
Gill 1 Hematoxylin	1 gal. (3.79 L) bottle	Ea.	72411
Gill 2 Hematoxylin	1 pt. (0.47 L) bottle	4/cs.	72504
Gill 2 Hematoxylin	1 gal. (3.79 L) bottle	Ea.	72511
Gill 3 Hematoxylin	1 pt. (0.47 L) bottle	4/cs.	72604
Gill 3 Hematoxylin	1 gal. (3.79 L) bottle	Ea.	72611
Modified Harris Hematoxylin	1 pt. (0.47 L) bottle	4/cs.	72704
Modified Harris Hematoxylin	1 gal. (3.79 L) bottle	Ea.	72711
Modified Mayer's Hematoxylin	1 pt. (0.47 L) bottle	4/cs.	72804
Differentiating Solution	1 pt. (0.47 L) bottle	4/cs.	74204
Differentiating Solution	1 gal. (3.79 L) bottle	Ea.	74211
OG-6	1 pt. (0.47 L) bottle	4/cs.	75204
OG-6	1 gal. (3.79 L) bottle	Ea.	75211
OG-6	2.5 gal. (9.46 L) cubitainer	Ea.	75225
Modified OG	1 gal. (3.79 L) bottle	Ea.	75311
EA-36	1 pt. (0.47 L) bottle	4/cs.	75404
EA-36	1 gal. (3.79 L) bottle	Ea.	75411
EA-50	1 pt. (0.47 L) bottle	4/cs.	75504
EA-50	1 gal. (3.79 L) bottle	Ea.	75511
EA-50	2.5 gal. (9.46 L) cubitainer	Ea.	75525
EA-65	1 pt. (0.47 L) bottle	4/cs.	75604
EA-65	1 gal. (3.79 L) bottle	Ea.	75611
EA-65	2.5 gal. (9.46 L) cubitainer	Ea.	75625
Modified EA	1 pt. (0.47 L) bottle	4/cs.	75704
Modified EA	1 gal. (3.79 L) bottle	Ea.	75711

