



Comparison of Hematoxylin and Eosin Stains on Automated Stainers

Instruction Manual

Purpose

This study was conducted to test the validity of automated H & E staining, on routinely formalin fixed and paraffin embedded prostate chips, colon and kidney tissue.

Principle

Differentiation is crucial for desired nuclear stain in regressive methods.

In progressive staining, immersion times are important to achieve good staining intensity.

Equipment

1. Sakura Autostainer - Prisma 81D
2. Sakura Autostainer Coverslipper
3. Leica Autostainer XL
4. Fisher Histomatic Autostainer

Technique

1. Paraffin sections were cut at 3 microns for slides with prostate chips, colon, and kidney samples
2. Slides with sections of colon, tonsil and placenta samples were cut at 4 microns
3. Skin samples were cut at 7 microns for frozen section
4. Slides were baked for 30 minutes in a 58°C oven

Reagents

- Xylene, Reagent Grade
- Alcohol, 95%, 100%
- 1N HCl to make 0.5% acid water
- Polysciences, Inc. Gill's #2 hematoxylin (cat. #24243)
- Polysciences, Inc. Gill's #3 hematoxylin (cat. #24244)
- Polysciences, Inc. Harris hematoxylin (cat. #24245)
- Polysciences, Inc. Eosin Y, 0.5% (cat. #09859)
- Polysciences, Inc. Eosin Y, 1.0% (cat. #17269)
- Polysciences, Inc. Ammonium Blue (cat. #24819)
- Polysciences, Inc. Lithium Blue (cat. #24820)
- Polysciences, Inc. Scott's Bluing Reagent (cat. #24605)
- Polysciences, Inc. Poly-Mount (cat. #08381)
- 0.045% Acid Alcohol (2600 ml of 70% alcohol and 1.2 ml of concentrated HCl)
- Ethyl alcohol
- 0.3% in-house ammonia water

Procedure for Automated Staining

1. Deparaffinize slides to water
2. Stain sections on automatic stainer
3. Times for Polysciences' Harris and Gill's hematoxylin were both the same for regressive stains
4. Coverslip slides

Procedure for Manual Staining

1. Fix frozen sections in 95% alcohol for 1 minute, then wash and air dry
2. Deparaffinize sections to water and air dry
3. Dip sections in water and stain in hematoxylin for 1 minute
4. Wash slides in water for about 20 dips
5. 10 dips in bluing reagent
6. Wash in water for 20 dips
7. Put slides in 95% alcohol for 10 dips
8. Treat slides with Eosin Y for 10 seconds
9. Dip 5-7 times in 95% alcohol
10. Two changes of absolute alcohol, 10 dips each
11. Two changes of xylene substitute, 10 dips each
12. Coverslip slides

Results

Stained nuclei appeared blue and cytoplasm was pink. Polysciences' Harris and Gill's hematoxylin needed 7-8 minutes and yielded good results. All Polysciences, Inc. bluing reagents demonstrated desirable results. Sections cut at 3 microns were stained lighter than those at 4-5 microns, which is ideal.

Table 1 Times for Sakura Autostainer - Prisma 81D

All buckets for the Sakura Autostainer - Prisma 81D hold 250 ml of reagent.

Step	Reagent	Polysciences' Harris/Gill's Regressive (time)	Polysciences' Gill's Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	1:00	1:00
4	0.5% Acid Alcohol	0:01	—
5	Wash	1:45	—
6	Bluing	0:30	0:30
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	1:00	1:30
10	95% OH	0:10	0:10
11	100% OH	0:10	0:10
12	100% OH	0:30	0:30
13	100% OH	1:30	1:30
14	Xylene	1:00	1:00
15	Xylene	1:00	1:00
16	Exit Xylene	1:00 - 3:00	1:00 - 3:00

Table 2 Times for Leica Autostainer XL

All buckets for the Leica Autostainer XL hold 450 ml of reagent.

Step	Reagent	Polysciences' Harris Regressive (time)	Polysciences' Gill's #2, #3 Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	1:00	1:00
4	0.045% Acid Alcohol	0:01	—
5	Wash	1:10	—
6	Bluing	0:30	0:30
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	2:30	2:30
10	95% OH	0:07	0:10
11	95% OH	0:10	0:10
12	100% OH	0:30	0:30
13	100% OH	1:00	1:00
14	100% OH	—	—
15	Xylene	1:00	1:00
16	Xylene	0:30	0:30
17	Exit Xylene	1:00 - 3:00	1:00 - 3:00

Table 3 Times for Fisher Histomatic Stainer

All buckets for Fisher Histomatic Autostainer hold 600 ml of reagent.

Step	Reagent	Polysciences' Harris Regressive (time)	Polysciences' Gill's #2, #3 Progressive (time)
1	Wash	0:10	0:10
2	Hematoxylin	8:00	2:00
3	Wash	0:50	0:50
4	0.045% Acid Alcohol	0:01	—
5	Wash	2:00	—
6	Bluing	0:20	0:20
7	Wash	1:00	1:00
8	95% OH	0:30	0:30
9	Eosin	2:00	3:00
10	95% OH	0:10	0:10
11	100% OH	0:30	0:30
12	100% OH	1:00	1:00
13	Xylene	1:00	1:00
14	Xylene	1:00	1:00

Table 4

Nuclear and Cytoplasmic Staining Intensity on the Sakura Autostainer - Prisma 81D

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin Y	Good
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, provided good contrast
Polysciences' Gill's #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Average
Polysciences' Gill's #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good, no bluish tinge
Polysciences' Gill's #3	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Good, no bluish tinge
Polysciences' Gill's #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Good

Table 5
Nuclear and Cytoplasmic Staining Intensity on Leica Autostainer XL

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin	Good combination with fresh acid alcohol
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Gill's	—	Polysciences' Ammonium Blue	Polysciences' Eosin	Good
Polysciences' Gill's	—	Polysciences' Lithium Blue	Polysciences' Eosin	Good, crisp with fresh acid
Polysciences' Gill's	—	Polysciences' Scott's Bluing	Polysciences' Eosin	Good, crisp stain

Table 6
Nuclear and Cytoplasmic Staining Intensity on Fisher Histomatic Autostainer

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Average
Polysciences' Gill's #2	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Good
Polysciences' Gill's #2	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent, crisp stain
Polysciences' Gill's #2	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent, crisp stain

Table 7
Nuclear and Cytoplasmic Staining Intensity with Manual
Regressive / Progressive Stain

Hematoxylin	Acid Alcohol	Blue	Eosin	Comments
Polysciences' Harris	0.045%	Polysciences' Ammonium Blue	Polysciences' Eosin	Excellent
Polysciences' Harris	0.045%	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Harris	0.045%	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Ammonium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Lithium Blue	Polysciences' Eosin Y	Excellent
Polysciences' Gill's #2, #3	—	Polysciences' Scott's Bluing	Polysciences' Eosin Y	Excellent

This study was conducted by Sakina Sadiq, B.S., HT (ASCP), HTL (ASCP) for Polysciences, Inc.

Material Safety Data Sheet

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Section 1: Chemical Product and Company Identification

Cat#: 09859

Part Name: EOSIN Y 0.5% ALCOHOLIC SOLUTION

 Supplier: Polysciences, Inc.
 400 Valley Road
 Warrington, PA 18976
 Telephone #215-343-6484

Section 2: Composition/ Information on Ingredients

Item#	Name	CAS#	% in product
1	Acetaldehyde	000075070	0.001
2	Acetic acid	000064197	0.6
3	Acetic acid, ethyl ester	000141786	0.7
4	Eosin Y	017372871	0.6
5	Ethyl alcohol	000064175	60
6	Methanol	000067561	3
7	Methyl isobutyl ketone	000108101	1
8	Water	007732185	32

OSHA (ACGIH) Exposure Limits

CAS#	IDLH	TWA		STEL		CEILING	
		ppm	mg/	ppm	mg/m3	ppm	mg/m3
CAS#: 000064175	IDLH: --						
OSHA		Not classifi	--	--	--	--	--
ACGIH		1000	1884	--	--	--	--
CAS#: 000064197	IDLH: 50						
OSHA		Not classifi	25	--	--	--	--
ACGIH		10	25	15	15	--	--
CAS#: 000067561	IDLH: 6,000						
OSHA		ACGIH;CA	260	--	--	--	--
ACGIH		200	262	250	250	--	--
CAS#: 000075070	IDLH: 2,000						
OSHA		Not classifi	360	--	--	--	--
ACGIH		--	--	--	--	25	45
CAS#: 000108101	IDLH: 500						
OSHA		CANADA	410	--	--	--	--
ACGIH		50	205	75	75	--	--
CAS#: 000141786	IDLH: 2,000						
OSHA		Not classifi	1400	--	--	--	--
ACGIH		400	1440	--	--	--	--
CAS#: 007732185	IDLH: NE						
OSHA		NE	NE	NE	NE	NE	NE
ACGIH		NE	NE	NE	NE	NE	NE
CAS#: 017372871	IDLH: NE						
OSHA		NE	NE	NE	NE	NE	NE
ACGIH		NE	NE	NE	NE	NE	NE

Section 3: Hazards Identification

Causes eye irritation.

Flammable liquid and vapor.

Harmful if swallowed.

Hazard Ratings:

These ratings are Polysciences' Inc. own assesments of the properties of the material using the ANSI/NFPA 704 Standard. Additional information can be found by consulting in the NFPA published ratings lists (List 325 and List 49).

If no data is listed the information is not available.

Health Flammability Reactivity

Section 4: First Aid Measures

Contact medical personnel immediately.
 Flush eyes with flowing water for at least 15 minutes.
 If swallowed, induce vomiting as directed by medical personnel.
 Separate eyelids with finger tips.
 Wash skin with deluge of water for at least 15 minutes.

Section 5: Fire Fighting Measures

Flash point, deg F.: 51 Method: TCC
 UEL: 19 LEL: 3.3 Autoignition temperature, deg. F.: no data
 Flammability Classification: IB Flame Propagation Rate: no data
 Hazardous Combustion Products: none

Section 6: Accidental Release Measures

Any information listed below is to be considered in addition to internal guidelines for isolation of spill, containment of spill, removal of ignition sources from immediate area, and collection for disposal of spill by trained, properly protected clean up personnel.
 Absorb liquids on absorbent material.
 Protect personnel from exposure.
 Remove ignition sources.
 Ventilate the area.

Section 7: Handling and Storage

Store at room temp

Section 8: Exposure Controls/ Personal Protection

The use of eye protection in the form of safety glasses with side shields and the use of skin protection for hands in the form of gloves are considered minimum and non-discretionary in work places and laboratories. Any recommended personal protection equipment or environmental equipment is to be considered as additional to safety glasses and gloves.
 Use latex or equivalent gloves.
 Use process enclosures, local exhaust ventilation, or other engineering controls.

Section 9: Physical and Chemical Properties

Formula:	no data	vapor pressure:	44.6 mm Hg
Formula Weight:	no data	vapor density:	1.6
boiling point:	173	Specific gravity:	0.86
melting point:	<-148	ph:	no data
solubility:	100%	appearance:	red colored liquid alcohol odor

Section 10: Stability and Reactivity

Chemical Stabilit stable
 Conditions to Avoid: none
 Incompatibility with other materials: none
 Hazardous Decomposition Products: none
 Hazardous Polymerization: will not occur

Section 11: Toxicological Information

Acute Data: no data
 Subchronic data: long term exposure may result in eye; liver and kidney damage

Section 12: Ecological Information

no data

Section 13: Disposal Considerations

The following chart lists the status of the chemical and its components in reference to 40 CFR Part 261.33. If the product is listed by code number the substance may be subject to special federal and state disposal regulations. If no codes are listed the material must be disposed in compliance with all Federal, State and Local Regulations.

CAS#	Waste Code	Regulated Name
000064175	not listed	not listed
000064197	not listed	not listed
000067561	U154	Methyl alcohol
000075070	U001	Ethanal

000108101	U161	Pentanol, 4-methyl-
000141786	U112	Ethyl acetate
007732185	not listed	not listed
017372871	not listed	not listed

Section 14: Transportation Data

Refer to bill of lading or container label for DOT or other transportation hazard classification , if any.

Section 15: Regulatory Information

All components of this product are on the TSCA public inventory.

Prop 65 - Column A identifies those items which are known to the State of California to cause cancer. Column B identified items which are known to the State of California to cause reproductive toxicity.

CAS#	Column A	Column B
000064175	no	no
000064197	no	no
000067561	no	no
000075070	Yes	no
000108101	no	no
000141786	no	no
007732185	no	no
017372871	no	no

State Regulatory Information :If a CAS# is listed below this material is subject to the listed state right-to-know requirements.

CAS#	FL	MA	MN	PA	WA	
000064175	FL	MA	MN	PA	WA	
000064197	FL	MA	MN	PA	WA	
000067561	FL	MA	MN	NJ	PA	WA
000075070	FL	MA	MN	NJ	PA	WA
000108101	FL	MA	MN	NJ	PA	WA
000141786	FL	MA	MN	PA	WA	
007732185	not listed					
017372871	not listed					

SARA Toxic Release Chemicals(as defined in Section 313 of SARA Title III)

This list identifies the toxic chemicals, including their de minimis concentrations for which reporting is required under Section 313 of the Emergency Planning and Community Right-to-Know Act (EPCRA). The list is also referred to as the Toxics Release Inventory (TRI) List.

CAS#	Regulated name	de minimis conc. %	Rep. Thres.
000064175	not listed	not listed	not listed
000064197	not listed	not listed	not listed
000067561	Methanol	1.0	not listed
000075070	Acetaldehyde	0.1	not listed
000108101	Methyl isobutyl ketone	1.0	not listed
000141786	not listed	not listed	not listed
007732185	not listed	not listed	not listed
017372871	not listed	not listed	not listed

SARA Extremely Hazardous Substances and TPQs

This list includes hazardous chemicals as defined in 29 CFR 1910.1200(c); and extremely hazardous substances regulated under Section 302 of SARA Title III with their TPQs (in pounds), as listed in 40 CFR 355, Appendices A and B.

CAS#	Regulated name	TPQ (pounds)	EHS-RQ(pounds)
000064175	not listed	not listed	not listed
000064197	not listed	not listed	not listed
000067561	not listed	not listed	not listed
000075070	not listed	not listed	not listed
000108101	not listed	not listed	not listed
000141786	not listed	not listed	not listed
007732185	not listed	not listed	not listed
017372871	not listed	not listed	not listed

CERCLA

The hazardous substances, and their reportable quantities (RQs) are listed in the federal regulations at 40 CFR Part 302, Table 302.4. Release of a CERCLA hazardous substance in an amount equal to or greater than its RQ, in any 24-hour period, must be reported to the National Response Center at (800) 424-8802.

CAS#	Regulated name	RQ (pounds)
000064175	Not listed	Not listed
000064197	Not listed	Not listed
000067561	Not listed	Not listed
000075070	Not listed	Not listed
000108101	Not listed	Not listed
000141786	Not listed	Not listed
007732185	Not listed	Not listed
017372871	Not listed	Not listed

Section 16: Other Information

POLYSCIENCES, INC. provides the information contained herein in good faith but makes no representation as to its comprehensiveness or accuracy.

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END OF MSDS