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Version 1

Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer V.1

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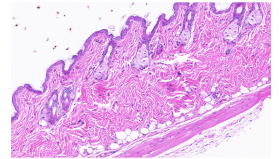
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Protocol status: Working

We use this protocol and it's working

Created: May 05, 2020

Last Modified: June 07, 2024

Protocol Integer ID: 36646

Keywords: hematoxylin, eosin, h&e, eosin protocol for leica st5020 automated stainer, leica st5020 automated stainer, eosin protocol, hematoxylin, automated stainer, leica st5020 automated multistainer, manual staining, protocol, own protocol

Abstract

This Hematoxylin and Eosin protocol is for the Leica ST5020 Automated multistainer. You can use it as a guide to developing your own protocol, catered to the needs of your researchers. This template can be modified for manual staining but best and most reproducible results are easier to achieve with an automated stainer.

Guidelines

Daily Maintenance for ST5020

	Filte r reag ent cup 21 (He mato xylin 560) usin g a funn el, Wha tman #1 filter pap er, and 500 ml Erle nme yer flask
	Disp ose of reag ent cup 1 (Pro - par) into aque ous wast e
	Mov e reag ent cup 2 into the 1 posit ion
	Mov e



	reag ent cup 3 into the 2 posit ion
	Fill a clea n, dry reag ent cup with fres h Pro- par and plac e in reag ent cup 3 posit ion
	Disp ose of reag ent cup 4 (100 %IP A) into aque ous wast e
	Mov e reag ent cup 5 into the 4 posit ion
	Fill a clea n,



	dry reag ent cup with fres h (100 %IP A) and plac e in reag ent cup 5 posit ion
	Disp ose of reag ent cup 6 into aque ous wast e and repl ace with fres h 90% IPA
	Disp ose of reag ent cup 33 (Defi ne) into aque ous wast e and repl ace with fres h Defi ne



	Dispose of reagent cup 32 (Blue Buffer 8) into aqueous waste and replace with fresh Blue Buffer 8 in a clean, dry reagent cup
	Dispose of reagent cup 31 (70 % IPA) into aqueous waste and replace with fresh 70% IPA
	Dispose of reagent cup



	29 (90 % IPA) into aque ous wast e and repl ace with fres h 90% IPA
	Disp ose of reag ent cups 16 and 17 (100 % IPA) into aque ous wast e
	Mov e reag ent cup 15 into the 17 posit ion
	Mov e reag ent cup 28 into the 16 posit ion
	Mov e reag



ent cup 27 into the 15 posit ion	
Fill clea n, dry reag ent cups with fres h 100 % IPA and plac e into the 27 and 28 posit ion	
Add appr oxim ately 20 gra ms of DriRi te to reag ent cups 27 and 28	
Disp ose of reag ent cup 26: (50: 50 Prop ar/10 0% IPA)	



	into aqueous waste
	Fill clean, dry reagent cup with 50:50 Propar/100% IPA. Add ~20 grams of DriRite and place in position 26
	Dispose of reagent cup 14 (Propar) into aqueous waste
	Move reagent cup 13 into the 14 position and add



	~20 grams of Dri- Rite
	Mov e reag ent cup 25 into the 13 posit ion
	Fill clea n, dry reag ent cup with fres h Pro- par and plac e in posit ion 25
	Rec ord main tena nce perf orm ed on the main tena nce shee t

Weekly Maintenance for ST5020

	Rem ove cups 7, 8, 9,
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10, 11, and 12	
Hand was h in hot soap y water r usin g a spon ge and Daw n dish dete rgen t	
Rins e thor oug hly in hot water	
Soa k cups in a 10% blea ch solut ion for ten minu tes	
Rins e thor oug hly in hot water	
Repl ace cups	



	into position
	Record maintenance performed onto the maintenance sheet

Monthly Maintenance for ST5020

	Dispose of reagent cup 21 (Hematoxylin 560) into aqueous waste
	Fill clean, dry reagent cup with fresh Hematoxylin 560 and place into the 21 position



	Disp ose of reag ent cup 18 (Eosi n Phlo xine 515) into aque ous wast e
	Fill clea n, dry reag ent cup with fres h Eosi n Phlo xine 515 and plac e into the 18 posit ion
	Rec ord main tena nce perf orm ed on the main tena nce shee t



Materials

MATERIALS

- ☒ Pro-Par Clearant **Anatech LTP Catalog #510**
- ☒ Isopropyl Alcohol 99% **Pacific Southwest Lab Equipment Catalog #104B**
- ☒ SelecTech Hematoxylin **Leica Biosystems Catalog #3801571**
- ☒ SelecTech Eosin 515 Phloxine **Leica Biosystems Catalog #3801606**
- ☒ SelecTech Define **Leica Biosystems Catalog #3803591**
- ☒ SelecTech Blue Buffer 8 **Leica Biosystems Catalog #3802916**

Solutions and Reagents:

1. Leica SelecTech Hematoxylin
2. Leica SelecTech Eosin 515 Phloxine
3. Leica SelecTech Define
4. Leica SelecTech Blue Buffer 8
5. Isopropyl Alcohol, 99%
6. Pro-Par Clearant

Prepare working Define solution by mixing 2 capfuls of Define filled to the fill line with 500 mls of deionized water.

Prepare working Blue Buffer 8 solution by mixing 2 capfuls of Blue Buffer 8 filled to the fill line with 500 mls of deionized water.

Hematoxylin and Eosin solutions are ready to use.

Equipment

ST5020	NAME
Automated Multistainer	TYPE
Leica	BRAND
Leica ST5020	SKU
https://www.leicabiosystems.com	LINK



H&E Stain

1

ST5020 Reagent Map

Pro-par	Pro-par	Pro-par	100%IPA	100%IPA	90%IPA	TAP Water	DI Water	DI Water	DI Water	DI Water	DI Water	DI Water
R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12	
Pro-par	Pro-par	100%IPA	100%IPA	100%IPA	SelecTech EOSIN Phloxine 515	Schiffs/ Acid/ Phosphomolybdic acid	Periodic Acid/ Biebrich Scarlett	SelecTech Hematoxylin 560	STATIC DI WATER	1% / 3% Acetic Acid		
R13	Dri-Rite R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24	
Pro-par	Dri-Rite 50/50 Propar 99IPA	100%IPA	100%IPA	90%IPA		70%IPA	SelecTech Define	SelecTech Blue Buffer 8	Weigerts Hematoxylin 3% acetic acid	LOAD	LOAD	
U25	R26	R27	R28	R29	R30	R31	R32	R33	R34	L35	L36	

Clip Colors

H&E

Depar

AB/PAS

Trichrome

PAS

rev 2020/08/08 AD

Reagent layout for multistain function

2

	STEP	STATION	DYE/REAGENT STATION	DURATION	EXACT	DIP
	1	38	Oven station	5:00	no	no
	2	37	Oven station	5:00	no	no
	3	1	Pro-Par	5:00	no	yes
	4	2	Pro-Par	5:00	no	yes
	5	3	Pro-Par	5:00	no	yes
	6	4	100% IPA	1:30	no	yes
	7	5	100% IPA	1:30	no	yes
	8	6	90% IPA	1:30	no	yes
	9	12	DI Water Wash	1:30	no	no



10	21	SelectTech Hematoxylin 560	1:30	yes	yes
11	11	DI Water Wash	1:30	no	no
12	33	SelectTech Define	1:00	yes	no
13	10	DI Water Wash	2:00	no	no
14	32	SelectTech Blue Buffer 8	1:30	yes	no
15	9	DI Water Wash	1:30	no	no
16	31	70% IPA	1:00	no	yes
17	18	ST Alcoholic Eosin Y 515	0:10	yes	yes
18	29	90% IPA	0:20	yes	yes
19	17	100% IPA	0:30	yes	yes
20	16	100% IPA	1:00	no	yes
21	15	100% IPA	1:30	no	yes
22	28	*100% IPA	1:30	no	yes
23	27	*100% IPA	1:30	no	yes
24	26	*50:50 Pro-par/100% IPA	1:00	yes	yes
25	14	*Pro-Par	5:00	no	yes
26	13	Pro-Par	5:00	no	yes
27	EXIT	Pro-Par			