VERSION 1
AUG 09, 2020

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.bf2ejqbe

Protocol Citation: Angela Denn 2020. Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bf2ejqbe>

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Protocol status: Working
We use this protocol and it's working

Created: May 05, 2020

Last Modified: Aug 09, 2020

PROTOCOL integer ID:
36646

Keywords: hematoxylin, eosin, h&e

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

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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

Filter reagent cup #1 (Hematoxylin 560) using a funnel
Whatman #1 filter paper, and 500ml Erlenmeyer flask
Dispose of reagent cup #1 (Proper) into aqueous waste

Move reagent cup 2 into the 1 position
Move reagent cup 3 into the 2 position
Fill a clean, dry reagent cup with fresh Pro-par and place in reagent cup 3 position
Dispose of reagent cup 4 (100% IPA) into aqueous waste
Move reagent cup 5 into the 4 position

	Fill a clean, dry reagent cup with fresh (100% IPA) and place in reagent cup 5 position
	Dispose of reagent cup 6 into aqueous waste and replace with fresh 90%IPA
	Dispose of reagent cup 33 (Define) into aqueous waste and replace with fresh Define

Dispo se of reage nt cup 32 (Blue Buffer 8) into aqueo us waste and replac e with fresh Blue Buffer 8 in a clean, dry reage nt cup	
Dispo se of reage nt cup 31 (70% IPA) into aqueo us waste and replac e with fresh 70% IPA	
Dispo se of reage nt cup 29 (90% IPA) into aqueo us waste and replac e with fresh 90% IPA	

Dispo se of reage nt cups 16 and 17 (100% IPA) into aqueo us waste
Move reage nt cup 15 into the 17 positi on
Move reage nt cup 28 into the 16 positi on
Move reage nt cup 27 into the 15 positi on
Fill clean, dry reage nt cups with fresh 100% IPA and place into the 27 and 28 positi on

Add approximately 20 grams of DriRite to reagent cups 27 and 28
Dispose of reagent cup 26 (50:50 Propar/100% 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
Move reagent cup 25 into the 13 position
Fill clean, dry reagent cup with fresh Propar and place in position 25
Record maintenance performed on the maintenance sheet

Weekly Maintenance for ST5020

Remove cups 7, 8, 9, 10, 11, and 12

	Hand wash in hot soapy water using a sponge and Dawn dish detergent
	Rinse thoroughly in hot water
	Soak cups in a 10% bleach solution for ten minutes
	Rinse thoroughly in hot water
	Replace cups into position
	Record maintenance performed onto the maintenance sheet

Monthly Maintenance for ST5020

Dispo se of reage nt cup 21 (Hem atoxyl in 560) into aqueo us waste	
Fill clean, dry reage nt cup with fresh Hema toxyli n 560 and place into the 21 positi on	
Dispo se of reage nt cup 18 (Eosin Phloxi ne 515) into aqueo us waste	
Fill clean, dry reage nt cup with fresh Eosin Phloxi ne 515 and place into the 18 positi on	

Record maintenance performed on the maintenance sheet

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

												O37	O38		
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	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	Schiffs/Phosphotungstic acid	Periodic Acid/Biebrich Scarlett	SelecTech Hematox 560	STATIC DI WATER	1% / 3% Acetic Acid	Alcian Blue				
R13	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 Hematox/ 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

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			
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