

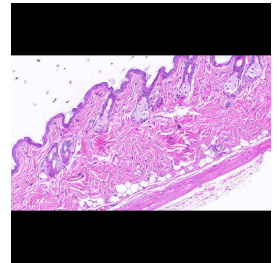
Nov 22, 2023

Version 2

Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer V.2

DOI

<https://dx.doi.org/10.17504/protocols.io.x54v9mozqg3e/v2>



Angela Denn¹

¹La Jolla Institute for Immunology

La Jolla Institute Micros...



Angela Denn

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.x54v9mozqg3e/v2>

Protocol Citation: Angela Denn 2023. Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.x54v9mozqg3e/v2> Version created by **Zbigniew Mikulski**



License: This is an open access protocol distributed under the terms of the **[Creative Commons Attribution License](#)**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: December 02, 2021

Last Modified: June 07, 2024

Protocol Integer ID: 55628

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

	Filter reagent cup 21 (Hematoxylin 560) using a funnel, Whatman #1 filter paper, and 500 ml Erlenmeyer flask
	Dispose of reagent cup 1 (Pro-par) into aqueous waste
	Move reagent cup 2 into the 1 position
	Move



	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 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)



	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,
--	-----------------------------------



	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 wate r
	Soa k cups in a 10% blea ch solut ion for ten minu tes
	Rins e thor oug hly in hot wate r
	Repl ace cups

	into posit ion
	Rec ord main tena nce perf orm ed onto the main tena nce shee t

Monthly Maintenance for ST5020

	Disp ose of reag ent cup 21 (He mato xylin 560) into aque ous wast e
	Fill clea n, dry reag ent cup with fres h Hem atox ylin 560 and plac e into the 21 posit ion



	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. 70%, 90%, and 100% Reagent Alcohol
6. Pro-Par Clearant

Prepare working Define solution by mixing 2 capfuls of Define filled to the fill line with 500 mls of 70% reagent alcohol.

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%RA	100%RA	90%RA	TAP Water	DI Water	DI Water	DI Water	DI Water	DI Water
R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11	R12
Xylene	Pro-par	100%RA	100%RA	100%RA	SelecTech EOSIN Phloxine 515	Schiffs/PPA/FGSR	Periodic/Biebrich/FG	SelecTech Hematox 560	STATIC DI WATER /Aniline Blue	1% / 3% 1% AA	Alcian Blue
R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24
	Propar	100%RA Dri-Rite	100%RA Dri-Rite	90%RA		70%RA	SelecTech Blue Buffer 8	SelecTech Define	Weigerts 3% AA/ 1% AA	LOAD	LOAD
U25	R26	R27	R28	R29	R30	R31	R32	R33	R34	L35	L36

Clip Colors

H&E

Depar Routine

AB/PAS

Trichrome

PAS

FG/SR

Depar IF

rev 2021/11/09 AD

Reagent layout for multi stainer use.

H&E Stain - Red clip

Step	Station	Reagent	Duration	Exact	Dip
1	38	Oven station	05:00	N	N
2	37	Oven station	05:00	N	N
3	1	Pro-par	05:00	N	Y
4	2	Pro-par	05:00	N	Y
5	3	Pro-par	05:00	N	Y
6	4	RA 100%	01:30	N	Y
7	5	RA 100%	01:30	N	Y
8	6	RA 90%	01:30	N	Y
9	12	Water station	01:30	N	N
10	21	Hematoxylin 560 SelecTech	01:30	Y	Y
11	11	Water station	01:30	N	Y
12	33	Define SelecTech	01:00	Y	N
13	10	Water station	02:00	N	N
14	32	Blue Buffer 8 SelecTech	01:30	Y	N
15	9	Water station	01:30	N	N
16	31	RA 70%	01:00	N	Y
17	18	Eosin Phloxine 515 SelecTech	00:10	Y	N
18	29	RA 90%	00:20	Y	N
19	17	RA 100%	00:30	Y	N
20	16	RA 100%	00:45	N	Y
21	15	RA 100%	00:45	N	Y
22	28	RA 100%	00:45	N	Y
23	27	RA 100%	00:45	N	Y
24	26	ProPar	02:00	N	Y
25	14	ProPar	05:00	N	Y
26	13	Xylene	03:00	N	Y
27	CV	CV1			

2021/05/06 AD

Program for H&E protocol.