

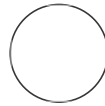
VERSION 2
NOV 22, 2023

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

Angela Denn¹

¹La Jolla Institute for Immunology

La Jolla Institute Microscopy Core



Angela Denn

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

OPEN ACCESS



DOI:
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](https://creativecommons.org/licenses/by/4.0/), 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

Filter
reage
nt cup
21
(Hem
atoxyl
in
560)
using
a
funnel
, What
man
#1
filter
paper,
and
500ml
Erlen
meyer
flask

Created: Dec 02, 2021

Last Modified: Nov 22, 2023

PROTOCOL integer ID: 55628

Keywords: hematoxylin, eosin, h&e

Dispo se of reage nt cup 1 (Pro- par) into aqueo us waste
Move reage nt cup 2 into the 1 positi on
Move reage nt cup 3 into the 2 positi on
Fill a clean, dry reage nt cup with fresh Pro- par and place in reage nt cup 3 positi on
Dispo se of reage nt cup 4 (100% IPA) into aqueo us waste
Move reage nt cup 5 into the 4 positi on

	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 (Pro-par) 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 Pro- par 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. 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

												O37	O38		
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	EOSIN Phloxine 515	Schiffs/PPA/FGSR	Periodic/Biebrich FG	SelecTech Hematox 560	STATIC DI WATER	1% / 3% AA					
R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24				
	Propar	100%RA	100%RA	90%RA		70%RA	SelecTech Blue Buffer 8	SelecTech Define	Weigerts 3% AA/1% AA	LOAD	LOAD				
U25	R26	Dri-Rite	Dri-Rite												
		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.

2

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.