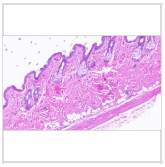


Aug 09, 2020

Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer

Angela Denn[1]

[1]La Jolla Institute for Immunology

1 Works for me dx.doi.org/10.17504/protocols.io.bf2ejqbe

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.

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

KEYWORDS

hematoxylin, eosin, h&e

CREATED

May 05, 2020

LAST MODIFIED

Aug 09, 2020

PROTOCOL INTEGER ID

36646

GUIDELINES

Daily Maintenance for ST5020

Filter reagent cup 21 (Hematoxylin 560) using a funnel, Whatman #1 filter paper, and 500ml Erlenmeyer flask
Dispose of reagent cup 1 (Pro-par) 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
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 aqueous waste and replace with fresh 90% IPA
Dispose of reagent cups 16 and 17 (100% IPA) into aqueous waste
Move reagent cup 15 into the 17 position
Move reagent cup 28 into the 16 position
Move reagent cup 27 into the 15 position
Fill clean, dry reagent cups with fresh 100% IPA and place into the 27 and 28 position
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

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
Dispose of reagent cup 18 (Eosin Phloxine 515) into aqueous waste
Fill clean, dry reagent cup with fresh Eosin Phloxine 515 and place into the 18 position
Record maintenance performed on the maintenance sheet

MATERIALS

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

MATERIALS TEXT

Solutions and Reagents:

Citation: Angela Denn (08/09/2020). Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer. https://dx.doi.org/10.17504/protocols.io.bf2ejqbe

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.

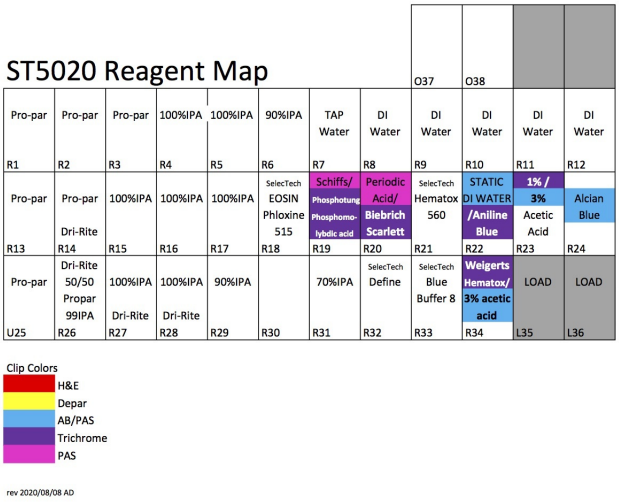
ST5020
Automated Multistainer
Leica Leica ST5020

EQUIPMENT

NAME	CATALOG #	VENDOR
ST5020	Leica ST5020	

H&E Stain

1



Reagent layout for multistain function

Citation: Angela Denn (08/09/2020). Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer. https://dx.doi.org/10.17504/protocols.io.bf2ejqbe

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			

Citation: Angela Denn (08/09/2020). Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer. https://dx.doi.org/10.17504/protocols.io.bf2ejqbe