

Jun 07, 2024

Fast Green/Sirius Red Protocol For Leica ST5020 Automated Stainer

 Forked from [Hematoxylin & Eosin Protocol For Leica ST5020 Automated Stainer](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.ewov19djklr2/v1>

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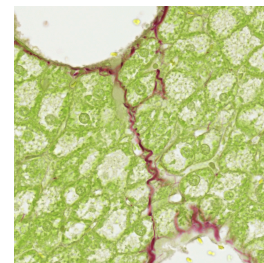
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Protocol Citation: Angela Denn, Zbigniew Mikulski 2024. Fast Green/Sirius Red Protocol For Leica ST5020 Automated Stainer. protocols.io <https://dx.doi.org/10.17504/protocols.io.ewov19djklr2/v1>



Manuscript citation:

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Protocol status: Working

We use this protocol and it's working

Created: June 07, 2024

Last Modified: June 07, 2024

Protocol Integer ID: 101393

Keywords: fibrosis, collagen, sirius red, picosirius, picosirius, fast green, staining, st5020, automated staining, histology, special stain, sirius red protocol for leica st5020 automated stainer, leica st5020 automated stainer, automated stainer, sirius red fast green protocol, sirius red protocol, special stain, manual staining, quality special stain, collagen in ffpe, demonstrating collagen, fast green, leica st5020 automated multistainer, frozen tissue, collagen, tissue

Abstract

This Sirius Red Fast Green 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 the best and most reproducible results are easier to achieve with an automated stainer.

This fork describes modifications to obtain high-quality special stain demonstrating collagen in FFPE and frozen tissues.

Image Attribution

La Jolla Institute for Immunology

Guidelines

Daily Maintenance for ST5020

A
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%RA) into aqueous waste.
Move reagent cup 5 into the 4 position.
Fill a clean, dry reagent cup with fresh (100%RA) and place in reagent cup 5 position.
Dispose of reagent cup 6 into aqueous waste and replace with fresh 90% RA.
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.
Dispose of reagent cup 31 (70% RA) into aqueous waste and replace with fresh 70% RA.
Dispose of reagent cup 29 (90% RA) into aqueous waste and replace with fresh 90% RA.
Dispose of reagent cups 16 and 17 (100% RA) 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% RA 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 (Propar) into aqueous waste.
Move reagent cup 14 (Pro-par) into the 26 position.
Fill a clean, dry reagent cup with fresh Pro-par and place into the 14 position.
Dispose of reagent cup 13 (Xylene) into aqueous waste.
Fill a clean, dry reagent cup with fresh xylene and place into the 13 position.
Record maintenance performed on the maintenance sheet.



Weekly Maintenance for ST5020

	A
	Remove cups 7, 8, 9, 10, 11, and 12.
	Hand wash in hot soapy water using a sponge and 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

	A
	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.
	Pour 1000ml of 10% bleach solution down drain.
	Pour 3000ml of hot tap water down drain.
	Calibrate touch screen.
	Record maintenance performed on the maintenance sheet.

Quarterly Maintenance for ST5020

Replace active carbon filter quarterly

Materials

MATERIALS

 Pro-Par Clearant **Anatech LTP Catalog #510**

 Direct-Red 80 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #365548**

 Reagent alcohol, 100% **Pacific Southwest Lab Equipment Catalog #374B**

 Xylene **Medical Chemical Corporation Catalog #134B-1gl**

 Fast Green FCF **Merck MilliporeSigma (Sigma-Aldrich) Catalog #F7252**

 Saturated Picric Acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P6744**

 Glacial acetic acid **Fisher Scientific Catalog #A38-500**

 Distilled Water

Solutions and Reagents:

1. Fast Green solution
2. Fast Green/Sirius Red solution
3. Acidified water
4. 90%, and 100% Reagent Alcohol
5. Pro-Par Clearant

Prepare Fast Green solution:

DI water *500 ml*

Fast Green *0.2 g*

Prepare Fast Green/Sirius Red solution:

Saturated picric acid *500 ml*

Fast Green *0.2 g*

Direct Red 80 *0.5 g*

Acidified Water:

DI water *1000 ml*

Glacial acetic acid *10 ml*

Equipment

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



Fast Green/Sirius Red Stain

- 1
- Perform all required maintenance on the stainer and prepare working solutions
- 2
- Arrange the reagents in the stainer according to the layout (note dark blue positions for FG/SR cups)

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	SelecTech EOSIN	Schiffs/ PPA/ FGSR	Periodic Biebrich FG	SelecTech Hematox 560	STATIC DI WATER /Aniline Blue	1%AA / 3%AA 1%AA	Toluidine Blue Alcian Blue Acid Water
R13	R14	R15	R16	R17	R18	R19	R20	R21	R22	R23	R24
	Propar	100%RA Dri-Rite	100%RA Dri-Rite	90%RA	Saf O / PSR / Cresyl Violet	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

Use white clip for all other programs

Reviewed 2022/08/23 AD

Reagent map for ST5020

- 3
- Program the FG/SR protocol into the ST5020 stainer according to the table below



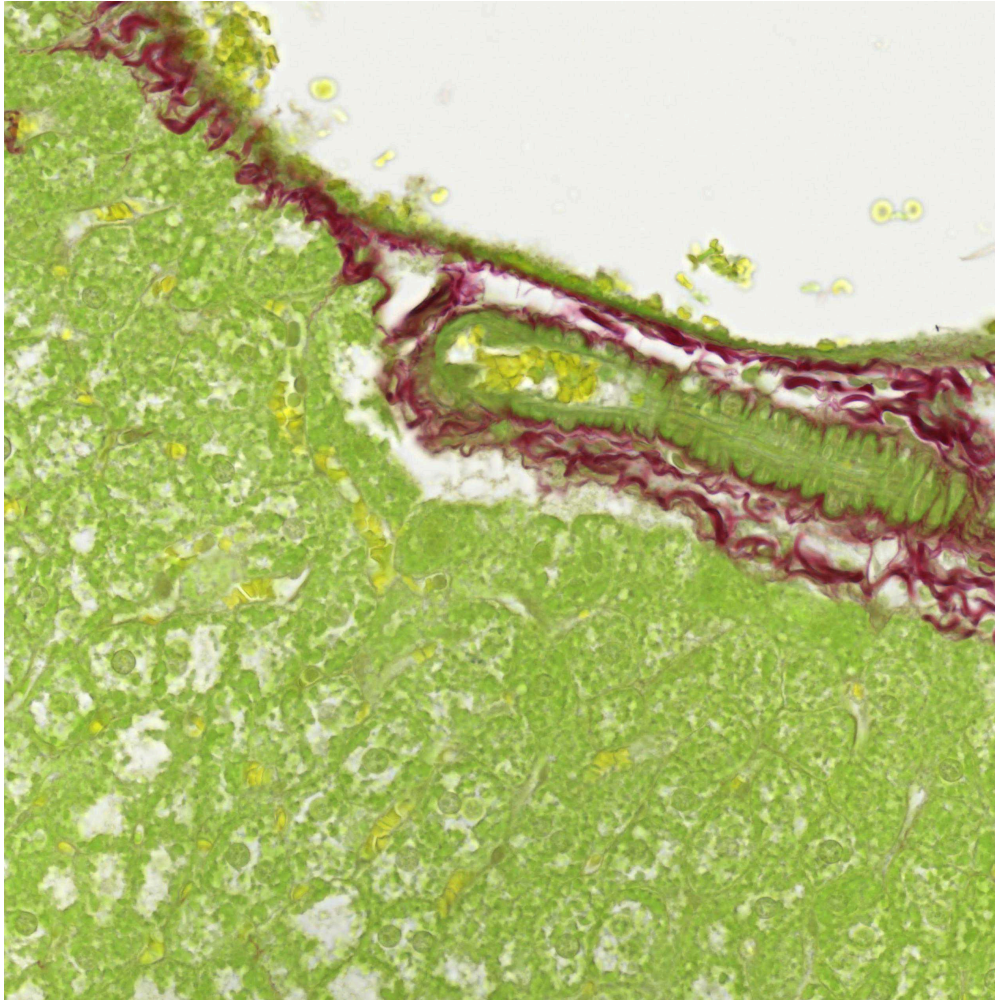
FGSR Stain – Dark blue clip

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	20	0.04% Fast Green Solution	15:00	Y	Y
11	11	Water station	01:30	N	Y
12	19	Fast Green/Sirius Red Solution	1:00:00	Y	N
13	34	Acidified water	00:30	Y	Y
14	23	Acidified water	00:30	Y	Y
15	17	Reagent Alcohol 100%	00:30	Y	N
16	16	Reagent Alcohol 100%	00:30	Y	N
17	15	Reagent Alcohol 100%	00:30	Y	Y
18	28	Reagent Alcohol 100%	00:30	Y	Y
19	27	Reagent Alcohol 100%	00:30	Y	Y
20	26	Pro-Par	02:00	N	Y
21	14	Pro-Par	05:00	N	Y
22	13	Xylene	02:00	N	Y
23	CV	CV Program 2			

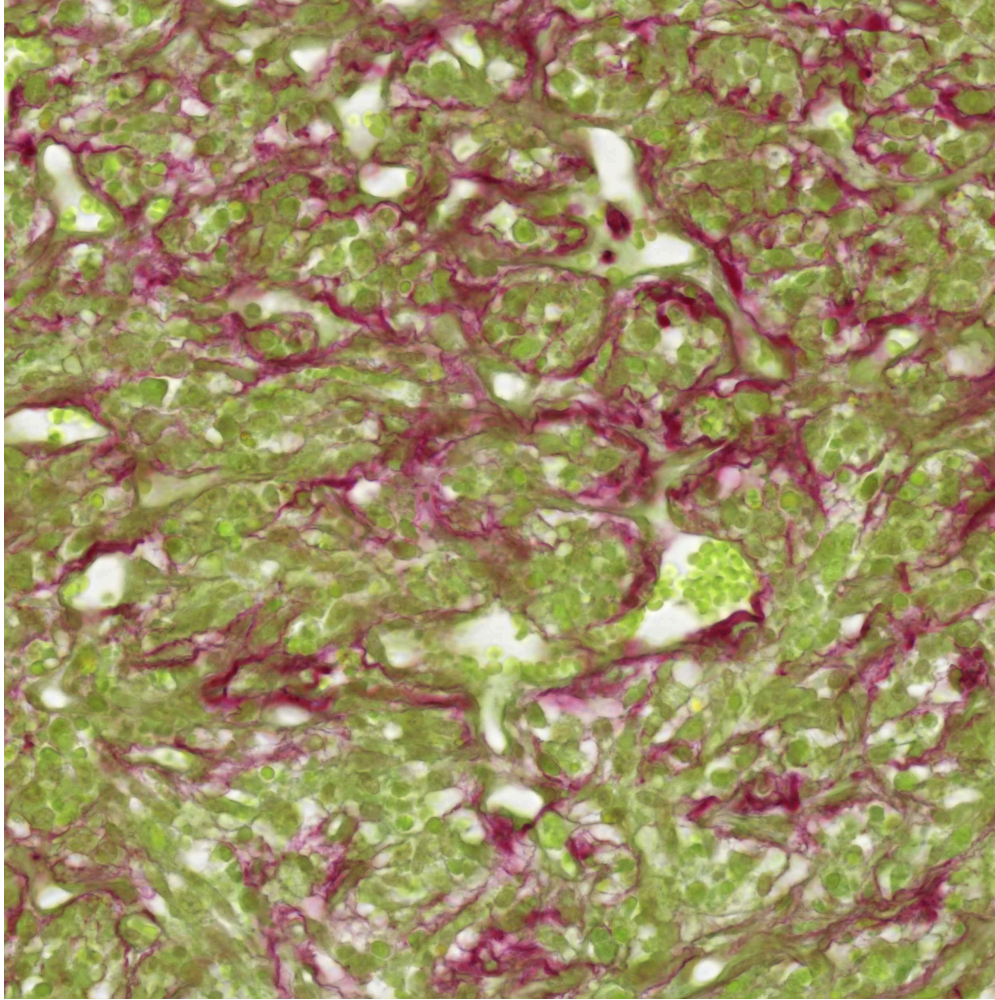
Steps for staining

Expected results

- 4 Collagen fibers should be stained red and all other non-collagen proteins should be stained green.



There is strong positive staining in collagen fibers along blood vessels and minimal red staining in other tissue areas. Normal mouse liver sample scanned with 40x 0.95NA objective with Zeiss AxioScan Z1



In this mouse tumor sample there are abundant collagen fibers stained red. Minimal background staining in other, non-collagenous areas of the tissue. Sample scanned with 40x 0.95NA objective with Zeiss AxioScan Z1

Protocol references

Segnani C, Ippolito C, Antonioli L, Pellegrini C, Blandizzi C, Dolfi A, Bernardini N. Histochemical Detection of Collagen Fibers by Sirius Red/Fast Green Is More Sensitive than van Gieson or Sirius Red Alone in Normal and Inflamed Rat Colon. PLoS One. 2015 Dec 16;10(12):e0144630. doi: 10.1371/journal.pone.0144630. PMID: 26673752; PMCID: PMC4682672.

<https://pubmed.ncbi.nlm.nih.gov/26673752/>