



# Affinity™ H&E Staining System

REF

ST5 & ST52

## Product Name

**Affinity™ H&E Staining System.** The Affinity™ H&E Staining System provides consistent staining quality in an easy 5 component kit. Each component is formulated to be Ready to Use right from the bottle. Each component is manually poured into staining dishes on automated stainer.

## Intended Use

The Affinity™ H&E Staining System is formulated to stain the nuclei and connective tissue for paraffin embedded specimens mounted on microscope slides. Nuclei are stained dark blue and connective tissue is stained varying shades of red, orange and pink. The H&E System does not detect or measure a marker or analyte. The Staining System can be used with any paraffin embedded human or animal specimen requiring histopathologic evaluation for the assessment of disease or pathology.

This system has been optimized for the Leica ST5010\* and ST5020\* stainers. \*(trademark of Leica Biosystems)

## Product Function

Staining results from use of the Affinity™ H&E Staining System assist with diagnosis under the microscope. Microscopic visualization of stained tissue sections enable interpretation by a trained professional. This *quantitative* interpretation, combined with results from other diagnostic test are used to render a medical diagnosis. The System functions through the attraction of charged components to oppositely charged molecules in cells

## Intended Use and application

Use by qualified laboratory personnel. The Affinity™ H&E Staining System is intended for in vitro diagnostics use only.

## Quality control

The Affinity™ H&E Staining System does not require calibration. It is recommended however, that control slides be included that contain tissue similar to patient specimens to ensure reagents are performing properly prior to staining diagnostic specimens.

## System Limitations

The Affinity™ H&E Staining System contains 5 specially formulated reagent components. The reagents have been formulated and optimized specifically on the Leica ST5010\* and ST5020\* stainers utilizing a tap water hook up and optimized for use with xylene and reagent grade alcohols which are not included.

## Applicable Products

Product Code	Description
ST5	Affinity™ H&E Staining System
ST52	Affinity™ 2 H&E Staining System
AST2	Hematoxylin
AST1	Pre-Stain
AST3	Differentiator
AST5	Eosin
AST4	Bluing

## Equipment required

The Affinity™ H&E Staining System was developed and optimized for use with the Leica ST5010\* and ST5020\* automated stainers.



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AutoFrost® SuperCoat™ Adhesive, AMS, IHC, and AutoFrost® non charged or equivalent slides should be used for best results. Prior to staining, slides should be dried at 58° - 60° C for 20 - 30 minutes.

### Storage and Stability

Store reagents at room temperature (15-30°C) and follow local guidelines for storage of flammable products. **CAUTION:** Do not use past expiration date.

The Affinity™ H&E Staining System has a slide capacity of 2,000 slides and 2 week stability. After staining 2,000 slides or after 2 weeks on the ST 5010\* or ST5020\* stainer (whichever occurs first) the reagents of the The Affinity™ H&E Staining System should be disposed in accordance with local governing regulations.

Reagent dishes should be covered to reduce evaporation and to maintain performance when reagents are not being used.

### Sterility

The Affinity™ H&E Staining System is **non-sterile**.

### Specimen Handling

Specimens stained with the Affinity™ H&E Staining System should be properly fixed and any general fixative including but not limited to neutral buffered formalin (10% NBF) may be used. Specimen should be processed, and paraffin embedded and sectioned at a standard thickness (2 – 5µm). Inadequate fixation, processing, and sectioning will compromise the staining quality.

Directions for use of the Affinity™ H&E Staining System on the ST5010 XL\* AutoStainer.

Settings for the ST5010 XL AutoStainer as well as a recommended reagent lay-out and protocol schedules are provided to optimize the performance and consistency of the Affinity™ H&E Staining System. Deviation from recommended protocol may result in compromised staining quality and capacity.

ST5010 AutoStainer XL: Bath Lay-Out

Reagent Lay-out for the ST5010 AutoStainer XL is shown below.

Empty entire contents of each of the bottles into the corresponding reagent dish and position. All of the reagents in each Affinity™ H&E Staining System Kit must be used together to achieve the expected staining performance.

Reagent substitutions that deviate from the kit can compromise performance.

Note: Reagents should be covered when not in use. Discard the staining reagents after two weeks or 2,000 slides whichever comes first

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ST5010 AutoStainer XL\*: Staining Protocol

**Figure 1. ST5010 Reagent Staining Dish Lay-Out**

1. xylene	2. xylene	3. xylene	4. 100% Reagent Alcohol	5. 100% Reagent Alcohol	6. 100% Reagent Alcohol	7. 80% Reagent Alcohol	Tap Water Wash 1	Affinity™ PRE- STAIN	Tap Water Wash 3	Tap Water Wash 4	Tap Water Wash 5	OVEN
EXIT xylene	18. xylene	17. xylene	16. xylene	15. 100% Reagent Alcohol	14. 100% Reagent Alcohol	13. 100% Reagent Alcohol	12. Affinity™ Eosin	11. 80% Reagent Alcohol	10. Affinity™ Bluing	9. Affinity™ Differentiator	8. Affinity™ Hematoxylin	LOAD

The Staining times and instrument settings for the ST5010 XL AutoStainer are detailed in Table 1. Three protocols for varying contrasts of the H&E stain are included in Table 1. The incubation times for the ST Hematoxylin and ST Eosin may be further varied within the parameters outlined in Table 2 to meet staining preferences. All other time requirements outlined in Table 1 must be adhered to in order to ensure quality and consistency of the staining results.

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Table 1. ST5010\* Staining Protocol

<b>Settings: Dips = 2, Withdrawal Speed = 9</b>					
Step	Station	Reagent	Time	Exact	
1	Load	N/A	0:00	N/A	
2	1	Xylene	2:00	NO	
3	2	Xylene	2:00	NO	
4	3	Xylene	2:00	NO	
5	4	100% Reagent Alcohol	1:00	NO	
6	5	100% Reagent Alcohol	1:00	NO	
7	6	100% Reagent Alcohol	1:00	NO	
8	7	80% Reagent Alcohol	1:00	NO	
9	Water Rinse 1	Water Rinse	1:00	NO	
10	Water Rinse 2*	Affinity™ Pre-Rinse	0:30	YES	
11	8	Affinity™ Hematoxylin	Protocol 1	2:00	YES
			Protocol 2	3:00	YES
			Protocol 3	5:00	YES
12	Water Rinse 5	Water Rinse	2:00	YES	
13	9	Affinity™ Differentiator	0:45	YES	
14	Water Rinse 4	Water Rinse	1:00	NO	
15	10	Affinity™ Bluing Agent	1:00	YES	
16	Water Rinse 3	Water Rinse	1:00	NO	
17	11	80% Reagent Alcohol	1:00	NO	
18	12	Affinity™ Eosin	Protocol 1	0:30	YES
			Protocol 2	0:50	YES
			Protocol 3	1:00	YES
19	13	100% Reagent Alcohol	1:00	YES	
20	14	100% Reagent Alcohol	1:00	YES	
21	15	100% Reagent Alcohol	1:00	YES	
22	16	Xylene	1:00	NO	
23	17	Xylene	1:00	NO	
24	18	Xylene	1:00	NO	

\*\*Replace Water Rinse station 2 with a standard reagent container. The opening within the rinse manifold should be sealed with a (screw) blocking plug (part number 14045635393) to prevent a loss of water pressure to the remaining water rinse stations." **credit Leica**

Staining times for Affinity™ Hematoxylin and Eosin may be modified within Table 2 (below) to achieve different contrasting staining preferences

Table 2: Affinity™ Hematoxylin and Eosin Variations

Reagent	Staining Time Range
Affinity™ Hematoxylin	2-5 min
Affinity™ Eosin	30-90 sec.

**Alcohol and Xylene Rotation**

The alcohols and xylenes used for deparaffinization, and rehydration should be rotated after every 600 slides. Reagent rotation is defined as follows:

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1. Empty the most contaminated reagent of a series and refill the container.
2. Move the remaining container(s) up one space in the sequence.
3. Place the newly filled container into the last position of the series.

The alcohols and xylenes used for dehydration and clearing before coverslipping also should be rotated after every 600 slides as described above.

The two 80% alcohols (steps 8 and 17) should be discarded and replaced with fresh 80% alcohol solutions after 600 slides. Failure to rotate and change reagents as advised may result in inadequate deparaffinization as well as excessive carry-over and dilution of reagents or solvents.

**Expected Results**

By following the instructions for use, a single Affinity Kit should provide at least 2,000 consistent high- quality stained slides.

**Directions for use:** Affinity H&E Staining System on the ST5020 Multistainer.

ST5020 Multistainer operating settings as well as staining dish layout and protocol schedule recommendations are provided to optimize the performance and consistency of the Affinity H&E Staining System.

Deviation from these instructions may result in compromised staining quality and capacity.

**ST 5020 Multistainer: Staining dish layout**

The required reagent staining dish layout for the ST5020 Multistainer is shown below in Figure 2.

Empty the entire contents of each of the bottles into the designated reagent dishes and positions. All of the reagents in each Affinity Kit must be used together to achieve the expected performance.

Substitution of non-Affinity reagents will potentially compromise staining performance.

Note: Reagent dishes should be covered when not in use to reduce evaporation. Discard the staining reagents if the minimal staining capacity (2000 slides) is not met within two weeks (14 days) after pouring into the reagent dishes.

**FIGURE 2. ST5020 Reagent Staining Dish Layout**

1. Xylene	2. Xylene	3. Xylene	4. 100% Reagent Alcohol	5. 100% Reagent Alcohol	6. 100% Reagent Alcohol	7. 80% Reagent Alcohol	8. Tap Water Rinse	9. Tap Water Rinse	10. Tap Water Rinse	11. Tap Water Rinse	12. Tap Water Rinse
13. Xylene	14. Xylene	15. 100% Reagent Alcohol	16. 100% Reagent Alcohol	17. 100% Reagent Alcohol	18. Affinity™ Eosin	19. 80% Reagent Alcohol	20. Affinity™ Bluing Agent	21. Affinity™ Differentiator	22. Affinity™ Hematoxylin	23. Affinity™ Pre-Stain	24.
U25. Xylene Exit	U26. Xylene Exit	U27. Xylene Exit	28. Xylene Exit	29.	30.	31.	32.	L33. Load	L34. Load	L35. Load	L36. Load

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**ST5020 Multistainer: Staining Protocol**

The staining times and instrument settings for the ST5020 Multistainer are included in Table 3. 3 protocols, each with different staining contrasts of the H&E stain are included. Staining times for the Affinity™ Hematoxylin and Eosin may be further differentiated within the parameters outlined in Table 4 to meet staining preferences. All other time guidelines outlined in Table 3 should be followed to ensure quality and consistency of staining.

**Table 3. ST5020 Staining Protocol**

Step	Station	Reagent	Time	Exact	
1	Load	N/A	0:00	N/A	
2	1	Xylene	2:00	NO	
3	2	Xylene	2:00	NO	
4	3	Xylene	2:00	NO	
5	4	100% Reagent Alcohol	1:00	NO	
6	5	100% Reagent Alcohol	1:00	NO	
7	6	100% Reagent Alcohol	1:00	NO	
8	7	80% Reagent Alcohol	1:00	NO	
9	Water Rinse 11	Water Rinse	1:00	NO	
10	23	Affinity™ Pre-Rinse	0:30	YES	
11	22	Affinity™ Hematoxylin	Protocol 1	2:00	YES
			Protocol 2	3:00	YES
			Protocol 3	5:00	YES
12	Water Rinse 10	Water Rinse	2:00	YES	
13	21	Affinity™ Differentiator	0:45	YES	
14	Water Rinse 9	Water Rinse	1:00	NO	
15	20	Affinity™ Bluing Agent	1:00	YES	
16	Water Rinse 8	Water Rinse	1:00	NO	
17	19	80% Reagent Alcohol	1:00	NO	
18	18	Affinity™ Eosin	Protocol 1	0:30	YES
			Protocol 2	0:50	YES
			Protocol 3	1:00	YES
19	17	100% Reagent Alcohol	1:00	YES	
20	16	100% Reagent Alcohol	1:00	YES	
21	15	100% Reagent Alcohol	1:00	YES	
22	14	Xylene	1:00	NO	
23	13	Xylene	1:00	NO	
24	Exit	Xylene	1:00	NO	

Staining times for Affinity™ Hematoxylin and Eosin may be modified within Table 4 (below) to achieve different contrasting staining preferences

**Table 4: Affinity™ Hematoxylin and Eosin Variations**

Reagent	Staining Time Range
Affinity™ Hematoxylin	2-5 min
Affinity™ Eosin	30-90 sec.

The alcohols and xylenes used for deparaffinization, and rehydration should be rotated after every 600 slides. Reagent rotation is defined as follows:



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1. Empty the most contaminated reagent of a series and refill the container.
2. Move the remaining container(s) up one space in the sequence.
3. Place the newly filled container into the last position of the series.

The alcohols and xylenes used for dehydration and clearing before coverslipping also should be rotated after every 600 slides as described above.

The two 80% alcohols (steps 8 and 17) should be discarded and replaced with fresh 80% alcohol solutions after 600 slides. Failure to rotate and change reagents as advised may result in inadequate deparaffinization as well as excessive carry-over and dilution of reagents or solvents.

### **Expected Results**

By following the instructions for use, a single Affinity™ Kit should provide at least 2,000 consistent high- quality stained slides.

### **Intended Use**

After the Affinity™ H&E Staining System is placed onto the ST5010 or ST5020 Stainer, the system is ready to be used. Place reagents in staining dishes and choose protocol.

### **Quality control**

The Affinity™ H&E Staining System does not require calibration. It is recommended however, that control slides be included that contain tissue similar to patient specimens to ensure reagents are performing properly prior to staining diagnostic specimens.

### **Product Function**

Staining results from use of the Affinity™ H&E Staining System assist with diagnosis under the microscope. Microscopic visualization of stained tissue sections enable interpretation by a trained professional. This *quantitative* interpretation, combined with results from other diagnostic test are used to render a medical diagnosis. The System functions through the attraction of charged components to oppositely charged molecules in cells

### **Intended Use and application**

Use by qualified laboratory personnel. The Affinity™ H&E Staining System is intended for in vitro diagnostics use only.

### **Disposal**

All reagents of the Affinity™ H&E Staining System should be disposed in accordance with local regulations.