Thermo
SCIENTIFIC

Thermo Scientific Phoenix Blue Nuclear Stain (Substitute for Hematoxylin) Instruction for Use

For in vitro diagnostic use.
For use as a nuclear stain.

Instructions For Use

Thermo Scientific™ Phoenix Blue™ Nuclear Stain stains the nuclei of cells purple to dark blue. It has been designed to be used as a nuclear stain (hematoxylin) substitute in the Hematoxylin and Eosin (H&E) stain. It can also be used as a nuclear stain for Cytology samples stained with the Papanicolaou stain, as well as a nuclear counterstain for immunohistochemistry (IHC).

Recommended protocols have been developed eliminating the Clarifier step; Phoenix Blue Nuclear Stain does not demonstrate significant background staining and is readily removed by acid rinses. Clarifier 1 is used in the recommended Cyto-Stain protocol to help define cytoplasmic hues but timing should be maintained at 30 seconds or less. Avoid use of Hydrochloric Acid solutions.

Note: Use of waterbath adhesives or charged/precoated slides may occasionally result in excessive background staining. Please contact the Laboratory Applications Team for specific instructions and corrective measures at 800-522-7270, ext. 562.

Phoenix Blue Nuclear Stain is manufactured in two stock solutions for enhanced stability; a working solution must be prepared prior to use. Once mixed, the Phoenix Blue Working Solution is stable for one week.

To Prepare Working Solution: Pour contents of Solution A into the bottle of Soultion B and mix thoroughly. The final volume will equal 500 mL. It is recommended to skim the top layer of the stain with a paper towel or laboratory wipe daily before using.

Protocol: Histology (Routine H&E Stain)

Station	Solution	Time (min:sec)
1	Clearing Reagent	3 minutes
2	Clearing Reagent	3 minutes
3	Clearing Reagent	3 minutes
4	100% Alcohol	1 minute
5	100% Alcohol	1 minute
6	100% Alcohol	1 minute
7	95% Alcohol	1 minute
8	Water Rinse	1 minute
9	Water Rinse	1 minute
10	Phoenix Blue Nuclear Stain Working Solution	2:30 to 4 minutes
11	Water Rinse	1 minute
12	Bluring Reagent	0:30 seconds
13	Water Rinse	1 minute
14	95% Alcohol	0:30 seconds
15	Eosin-Y	0:30 seconds
16	100% Alcohol	1 minute
17	100% Alcohol	1 minute
18	100% Alcohol	1 minute
19	Clearing Reagent	1 minute
20	Clearing Reagent	1 minute
21	Clearing Reagent	1 minute

Protocol: Cytology (Papanicolaou Stain using OG & EA)

Station	Solution	Time (min:sec)
1	95% Alcohol	3 minutes
2	95% Alcohol	2 minutes
3	Deionized Water	0:30 seconds
4	Deionized Water	0:30 seconds
5	Phoenix Blue Nuclear Stain Working Solution	2 to 3 minutes
6	Deionized Water	0:30 seconds
7	Bluring Reagent	0:30 seconds
8	Deionized Water	0:30 seconds
9	95% Alcohol	0:30 seconds
10	OG-6	1 to 2:30 seconds
11	95% Alcohol	0:15 to 1 minute
12	95% Alcohol	0:15 to 1 minute
13	EA-50	1 to 2:30 seconds
14	95% Alcohol	0:15 to 1 minute
15	95% Alcohol	0:15 to 1 minute
16	100% Alcohol	0:30 seconds
17	100% Alcohol	0:30 seconds
18	100% Alcohol	0:30 seconds
19	Clearing Reagent	0:30 seconds
20	Clearing Reagent	0:30 seconds
21	Clearing Reagent	0:30 seconds

Protocol: Cytology (Papanicolaou Stain using Cyto-Stain)

Station	Solution	Time (min:sec)
1	95% Alcohol	3 minutes
2	95% Alcohol	2 minutes
3	Deionized Water	0:30 seconds
4	Deionized Water	0:30 seconds
5	Phoenix Blue Nuclear Stain Working Solution	2 to 3 minutes
6	Deionized Water	0:30 seconds
7	Clarifier 1	0:00 to 0:30 seconds
8	Deionized Water	0:30 seconds
9	Bluing Reagent	0:30 seconds
10	Deionized Water	0:30 seconds
11	95% Alcohol	0:30 seconds
12	Cytostain	0:30 to 1:30 seconds
13	95% Alcohol	0:15 to 1 minute
14	95% Alcohol	0:15 to 1 minute
15	100% Alcohol	0:30 seconds
16	100% Alcohol	0:30 seconds
17	100% Alcohol	0:30 seconds
18	Clearing Reagent	0:30 seconds
19	Clearing Reagent	0:30 seconds
20	Clearing Reagent	0:30 seconds
21	Clearing Reagent	0:30 seconds

Protocol: Nuclear Counterstain for Immunohistochemistry

Station	Solution	Time (min:sec)
1	Perform IHC per Laboratory SOP	Variable
2	Rinse slides with Deionized Water	1 minute
3	Phoenix Blue Nuclear Stain Working Stain	1:30 to 2 minutes
4	Deionized Water	1 minute
5	DAB Chromogen: Dehydrate, Clear, Mount w/ Permanent Mountant (per Laboratory SOP)	Variable
5	AEC or Fast Red Chromogens: Mount w/Aqueous Mountant (per Laboratory SOP)	Variable

Instruction for use in Thermo Scientific Shandon Rapid Chrome Staining Kits and Thermo Fisher Scientific Chromoview Special Stain Kits

Phoenix Blue Nuclear Stain Working Solution

Combine 17 mL of Phoenix Blue Solution A and 33 mL of Phoenix Blue Solution B. Mix well. Solution is stable for one week at room temperature.

Protocol: Rapid Chrome H&E Frozen Section Stain

Station	Solution	Time
1	Rapid Fixx™	5–7 seconds
2	Distilled Water	5–10 dips
3	Phoenix Blue Nuclear Stain Working Solution	2–3 minutes
4	Distilled Water	5–10 dips
5	Bluing Reagent	3 dips
6	95% Alcohol	5–7 dips
7	Eosin-Y	15 seconds
8	95% Alcohol	5–7 dips
9	100% Alcohol	5–7 dips
10	100% Alcohol	5–7 dips
11	Xylene	5–7 dips
12	Xylene	5–7 dips

Continued on next page.

Protocol: Rapid Chrome Papanicolaou Stain

Station	Solution	Time (min:sec)
1	95% Alcohol	1 minute
2	95% Alcohol	10 dips
3	Distilled Water	10 dips
4	Phoenix Blue Nuclear Stain Working Solution	1 – 2 minutes
5	Distilled Water	10 dips
6	Bluing Reagent	1 minute
7	Distilled Water	10 dips
8	95% Alcohol	10 dips
9	OG-6	1 minute
10	95% Alcohol	10 dips
11	95% Alcohol	10 dips
12	EA-50	1 minute
13	95% Alcohol	10 dips
14	95% Alcohol	10 dips
15	100% Alcohol	10 dips
16	100% Alcohol	10 dips
17	100% Alcohol	10 dips
18	Xylene	10 dips
19	Xylene	1 minute

Amyloid Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Modified Mayer's Hematoxylin)

Phoenix Blue Solution A	17 ml
Phoenix Blue Solution B	33 ml

Mix Well.
Solution is stable for one week at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Stain sections in Working Alkaline Congo Red Solution for 20 minutes.
3. Rinse sections in distilled water for 1 minute.
4. Stain sections in Working Phoenix Blue for 3 minutes.
5. Rinse sections in distilled water for 1 minute.
6. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
7. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results
Amyloid – Red to Pink-Red
Nuclei – Blue
Elastic Fibers – Light red
Amyloid – Polarized light – Apple Green

Gomori Trichrome (Blue Collagen) Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Weigert's Iron Hematoxylin)

Phoenix Blue Solution A	17 mL
Phoenix Blue Solution B	33 mL

Mix Well.
Solution is stable for one week at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Bouin's Fluid at 56° C for 1 hour.
3. Rinse sections in running tap water for 3-5 minutes until yellow color is removed.
4. Place sections in Working Phoenix Blue for 5 minutes at room temperature.
5. Rinse sections in running tap water for 1 minute.
6. Stain sections in Trichrome stain for 15 minutes.
7. Rinse sections in several changes of distilled water.
8. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
9. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results
Nuclei – Blue to Black
Cytoplasm and Muscle Fibers – Red
Collagen – Blue

Gomori Trichrome (Green Collagen) Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Weigert's Iron Hematoxylin)

Phoenix Blue Solution A	17 mL
Phoenix Blue Solution B	33 mL

Mix Well.
Solution is stable for one week at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Bouin's Fluid at 56° C for 1 hour.
3. Rinse sections in running tap water for 3-5 minutes until yellow color is removed.
4. Place sections in Working Phoenix Blue for 5 minutes at room temperature.
5. Rinse sections in running tap water for 1 minute.
6. Stain sections in Trichrome stain for 15 minutes.
7. Rinse sections in several changes of distilled water.
8. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
9. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results
Nuclei – Blue to Black
Cytoplasm and Muscle Fibers – Red
Collagen – Green

Masson Trichrome Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Weigert's Iron Hematoxylin)

Phoenix Blue Solution A	17 mL
Phoenix Blue Solution B	33 mL

Mix Well.
Solution is stable for one week at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Bouin's Fluid at 56° C for 1 hour.
3. Rinse sections in running tap water for 3-5 minutes until yellow color is removed.
4. Place sections in Working Phoenix Blue for 5 minutes at room temperature.
5. Rinse sections in running tap water for 1 minute.
6. Stain sections in Biebrich Scarlet-Acid Fuchsin for 5 minutes.
7. Rinse sections in distilled water for 30 seconds.
8. Place sections in Phosphotungstic-Phosphomolybdic Acid Solution for 5 minutes. Do not rinse.
9. Place sections in Aniline Blue Stain solution for 5 minutes.
10. Rinse sections in distilled water for 30 seconds.
11. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
12. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Note: The substitution of Working Phoenix Blue for Weigert's Iron Hematoxylin will result in nuclear detail appearing red to red violet.

Results
Nuclei – Red to Red Violet
Cytoplasm and muscle fibers – Red
Collagen – Blue

Mucicarmine Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Weigert's Iron Hematoxylin)

Phoenix Blue Solution A	17 mL
Phoenix Blue Solution B	33 mL

Mix Well.
Solution is stable for one week at room temperature.

Working Mucicarmine Solution

Mix one part Mucicarmine Stock Solution with four parts tap water.
Solution will last up to 3-4 days if refrigerated at (2-8° C).

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Working Phoenix Blue for 5 minutes at room temperature.
3. Rinse sections in several changes of distilled water.
4. Stain sections in Working Mucicarmine for 30 minutes.
5. Rinse sections in distilled water for 1 minute.
6. Stain sections in Tartrazine Stain Solution for 5 dips.
7. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
8. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results
Nuclei – Black to Blue
Cryptococci Capsule – Red
Mucin – Red
Background - Yellow

Continued on next page.

Periodic Acid-Schiff Special Stain Kit

Phoenix Blue Nuclear Stain Working Solution
(Substitute for Hematoxylin 1)

Phoenix Blue Solution A	17 mL
Phoenix Blue Solution B	33 mL

Mix Well.
Solution is stable for one week at room temperature.

Standard Staining Protocol

1. Deparaffinize and hydrate sections to distilled water.
2. Place sections in Periodic Acid for 5 minutes at room temperature.
3. Rinse sections in several changes of distilled water.
4. Stain sections in Schiff reagent for 15 minutes to achieve desired contrast.
5. Rinse sections in lukewarm running tap water for 10 minutes.
6. Stain sections in Working Phoenix Blue for 3 minutes.
7. Rinse sections in distilled water for 30 seconds.
8. Dehydrate sections in two changes of anhydrous alcohol for 1 minute each.
9. Clear sections in three changes of clearing reagent for 1 minute each and mount.

Results
Carbohydrates, Glycogen, Basement Membranes, Fungus – Magenta
Nuclei – Blue
Background – Light Purple

Warnings and Precautions

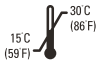
See Safety Data Sheets for warnings and precautions, as well as EUH code definitions.
See container label for warnings and precautions.

Order Information

Product		Qty.	REF
Phoenix Blue Nuclear Stain*	cs.	7214	

*Case contains two 170 mL bottles of Solution A and two 330 mL bottles of Solution B. When mixed together, one each of Solution A and B will yield 500 mL.

Anatomical Pathology



IS8142 R10/15

Richard-Allan Scientific
Subsidiary of Thermo Fisher Scientific
4481 Campus Drive, Kalamazoo, MI 49008
Phone: 1 (800) 522-7270
Phone: + 1 (269) 544-5600
www.thermoscientific.com/pathology

Thermo Shandon Limited
Subsidiary of Thermo Fisher Scientific
Tudor Road, Manor Park,
Runcorn, Cheshire WA7 1TA, UK
Phone: +44 (0) 1928 534 050
Fax: + 44 (0) 1928 534 049

CE
IVD

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand