

Eosin-Phloxine Stock Stain Set - Technical Memo

SET INCLUDES:

Solution A: Eosin Y Stock Stain Solution, 1% Aqueous (1 liter)
Solution B: Phloxine B Stock Stain Solution, 1% Aqueous (100 ml)

Part 1082A

Additionally Needed For Eosin-Phloxine Stock Stain Set:

Alcohol, Ethyl Denatured, 95% Part 10842
Acetic Acid Glacial, ACS Part 10010

Additionally Needed For H&E Staining:

Xylene, ACS	Part 1445		
Alcohol, Ethyl Denatured, 100%	Part 10841		
Alcohol, Ethyl Denatured, 95%	Part 10842		
Hematoxylin Stain Solution, Harris Modified	Part 1201	or	Hematoxylin Stain Solution, Harris Non-Mercuric Part 12013
Acid Alcohol, 1% Solution	Part 10011		
Lithium Carbonate Saturated Aqueous Solution	Part 12215	or	Scott Tap Water Substitute Part 1380
Alcohol, Ethyl Denatured, 70%	Part 10844		

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

The routine hematoxylin and eosin (H&E) stain is used for screening specimens in anatomic pathology, as well as for research, smears, touch preps and other applications. Its two primary coloring agents stain all cellular material including nuclei (blue), and cytoplasmic elements (pink-red). Popularity of this stain is due, in large measure, to its simplicity, ability to clearly demonstrate a wide variety of different tissue components, dependability, repeatability, and speed of use.

Eosin's particular value in the hematoxylin and eosin stain (H&E) is in its ability to distinguish between the cytoplasm of different types of cells by staining cytoplasmic components differing shades and intensities of pink to red.

Newcomer Supply Eosin-Phloxine Stock Stain Set solutions are aqueous based and intended to give an extra fine touch to routine H&E stains. Differentiation of muscle, connective tissue and epithelial elements tend to be sharper and demonstrate better than with an eosin solution alone.

METHOD:

Fixation: 10% Phosphate Buffered Formalin (Part 1090)

Technique: Paraffin sections cut at 5 microns

Solutions: All solutions are manufactured by Newcomer Supply, Inc.

Standard Working Solution:

Solution A: Eosin Y Stock Stain Solution, 1% Aqueous	100 ml
Solution B: Phloxine B Stock Stain Solution, 1% Aqueous	10 ml
Alcohol, Ethyl Denatured, 95%	780 ml
Acetic Acid Glacial, ACS	4 ml

H&E STAINING PROCEDURE WITH EOSIN-PHLOXINE:

- Deparaffinize sections thoroughly in three changes of xylene, 3 minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - See Procedure Notes #1 and #2.
- Stain with Hematoxylin Stain Solution, Harris Modified (1201) or Harris Non-Mercuric (12013) 1 to 5 minutes, depending on preference of nuclear stain intensity.
- Wash well in three changes of tap water.
- Differentiate quickly in Acid Alcohol, 1% Solution.
 - See Procedure Note #3.
- Rinse in three changes of tap water.
- Blue slides in Lithium Carbonate Saturated Aqueous Solution (12215) or Scott Tap Water Substitute (1380) for 10 dips.

- Wash in three changes of tap water; rinse in distilled water.
- Drain excess water from staining rack/slides; proceed to 70% alcohol for 10 dips.
- Counterstain in Eosin-Phloxine Standard Working Solution for 30 seconds to 3 minutes, depending on preference of intensity.
- Dehydrate in two changes of 95% ethyl alcohol for 1 minute each and two changes of 100% ethyl alcohol, 10 dips each. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

Nuclei	Blue
Erythrocytes and eosinophilic granules	Bright pink to red
Cytoplasm and other tissue elements	Various shades of pink

PROCEDURE NOTES:

- Drain staining rack/slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during staining procedure.
- Differentiate for a length of time to suit preference of nuclear stain intensity. Check slides microscopically to assure hematoxylin intensity is satisfactory. Nuclei should be distinct and the background very light to colorless.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 126-127.
- Carson, Freida. *Histotechnology: A Self-Instructional Text*. 2nd ed. Chicago: ASCP Press, 1997. 94-96.
- Luna, Lee G. *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Gaithersburg, MD: American Histolabs, 1992. 86-87, 91-92.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 143-144, 153-154.
- Modifications developed by Newcomer Supply Laboratory.

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product Name: Eosin-Phloxine Stain Set, Sol'n A: Eosin Y Stock Stain 1%, Aqueous
Part Number: 1082
CAS-No.: Not applicable
SDS Number: 2790

1.2 Recommended Use: Laboratory Chemicals

1.3 Company: Newcomer Supply
 2505 Parview Road
 Middleton, WI 53562 USA
Telephone: 1-800-383-7799
Fax: 1-608-831-0866
Website: www.newcomersupply.com
Email: newly@newcomersupply.com

24 HOUR EMERGENCY CONTACT
 CALL CHEMTREC: 1-800-424-9300
 Contact CHEMTREC only in the event of an emergency involving a chemical spill, leak, fire, exposure or other accident.

2. HAZARD(S) IDENTIFICATION

2.1 Classification of the substance or mixture
 GHS Classification, (in accordance with 29 CFR1910.1200)
 Serious Eye Damage/Eye irritation, Category 2A

2.2 GHS Label elements

Signal Word WARNING

Pictogram



Hazard Statement(s):

- Causes serious eye irritation

Precautionary Statement(s):

Prevention:

- Wash skin thoroughly after handling
- Wear protective gloves/protective clothing/eye protection/face protection

Response:

- IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing
- If eye irritation persists get medical advice/attention

2.3 Description of any hazards not otherwise classified None

2.4 >1% of mixture with unknown acute toxicity None

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.2 Mixture
Hazardous Components

Component		Concentration
Name	Eosin Y Powder	
CAS-No.	17372-87-1	1%

4. FIRST-AID MEASURES
4.1 Description of necessary measures
Inhalation (breathing)

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Get medical advice/attention if you feel unwell.

Skin Contact

Version 1.1

Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse. If skin irritation occurs: Get medical advice/attention.

Eye Contact

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. If eye irritation persists get medical advice/attention.

Ingestion (swallowed)

IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. Call a POISON CENTER or doctor/physician.

4.2 Most important symptoms and or effects, acute and delayed

The most important symptoms/effects are presented in Section 2 and or Section 11.

4.3 Indication of any immediate medical attention and special treatment needed

No data available

5. FIRE-FIGHTING MEASURES
5.1 Suitable extinguishing media

Carbon dioxide, dry chemical, water spray, alcohol-resistant foam.

5.2 Specific hazards arising from the substance or mixture

No data available

5.3 Protective equipment and precautions for fire-fighters

Wear a positive-pressure self-contained breathing apparatus if necessary. Wear chemical resistant clothing as recommended by clothing manufacturer.

NFPA Rating

Health	Fire	Reactivity
hazard: 0	hazard: 0	hazard: 0

6. ACCIDENTAL RELEASE MEASURES
6.1 Personal precautions, protective equipment and emergency procedures

Apply personal protective equipment (see Section 8). Use in a properly ventilated area. Avoid breathing vapors. Avoid skin contact. Avoid eye contact. Wash hands after use.

6.2 Methods and material for containment and cleaning up

Apply personal protective equipment (see Section 8). Contain spill. Prevent further leakage if possible and safe to do so. Ensure proper ventilation. For small amounts, wipe or absorb spill using inert material and dispose of according to local regulations. For large amounts, evacuate area and limit access. Prevent entry of material into sewage drains and confined areas. Dispose of any contaminated materials according to local regulations.

7. HANDLING AND STORAGE
7.1 Precautions for safe handling

Do not breathe dust/fume/gas/mist/vapours/spray. Do not get in eyes, on skin, or on clothing.

7.2 Conditions for safe storage, including any incompatibilities

Refer to Section 2.2 for proper storage temperature. Store the tightly closed container in a cool, dry, well-ventilated area.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION
8.1 Control Parameters

Does not contain components with occupational exposure limits.

8.2 Exposure Controls

Appropriate engineering controls

Version 1.1

Use in a properly ventilated area. Remove/wash before reuse contaminated clothing. Wash hands upon exiting work premises.

8.3 Personal Protective Equipment
Eye/Face protection

Wear chemical safety goggles and/or a full face shield if splashing is possible. Keep eye wash fountain nearby.

Skin Protection

Wear chemical-resistant gloves. Gloves should be resistant to components of product. Refer to glove manufacturer for appropriate type and glove thickness.

Body Protection

No data available

Respiratory Protection

No data available

Other Information

None

9. PHYSICAL AND CHEMICAL PROPERTIES
9.1 Information on basic physical and chemical properties

Physical state	Deep red liquid
Odor	Odorless
Odor threshold	No data available
pH	No data available
Melting point/freezing point	ca. 0°C (ca. 32°F)
Initial boiling point and boiling range	ca. 100°C (ca. 32°F)
Flash point	No data available
Evaporation rate	No data available
Flammability (solid, gas)	No data available
Upper flammability or explosive limits	No data available
Lower flammability or explosive limits	No data available
Vapor pressure	No data available
Vapor density	No data available
Relative density	Similar to water
Solubility(ies)	Water soluble
Partition coefficient: n-octanol/water	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	No data available

10. STABILITY AND REACTIVITY
10.1 Reactivity

No data available

10.2 Chemical stability

Stable in a closed container within label-specified storage temperature and expiration date.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

No data available

10.5 Incompatible materials

Version 1.1

Strong oxidizing agents

10.6 Hazardous decomposition products

No data available

11. TOXICOLOGICAL INFORMATION**11.1 Information on toxicological effects****Inhalation exposure**

No data available

Oral exposure

No data available

Dermal exposure

No data available

Skin corrosion/irritation

No data available

Serious eye damage/irritation

No data available

Respiratory or skin sensitization

No data available

Germ Cell mutagenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

Acute toxicity

LD50 mouse oral 2344 mg/kg

Carcinogenicity

IARC: None of the components are listed

NTP: None of the components are listed

OSHA: None of the components are listed

Additional information

RTECS: No data available

12. ECOLOGICAL INFORMATION**12.1 Ecotoxicity**

No data available

12.2 Persistence and degradability

No data available

12.3 Bioaccumulative potential

No data available

Version 1.1

12.4 Mobility in soil
No data available

12.5 Other adverse effects
No data available

13. DISPOSAL CONSIDERATIONS**13.1 Waste disposal methods****Contents**

Dispose of contents in a safe manner to comply with local, state and federal regulations. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging

Dispose of packaging in a safe manner to comply with local, state and federal regulations. Contact a licensed professional waste disposal service to dispose of this material.

14. TRANSPORT INFORMATION**14.1 DOT (US)**

UN-Number	No data available
Proper shipping name	No data available
Hazard class	No data available
Packing group	No data available
Environmental hazards	No data available

15. REGULATORY INFORMATION

15.1 No data available

16. OTHER INFORMATION

Preparation Information

Newcomer Supply Inc.

800-383-7799

www.newcomersupply.com

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1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product Name: Eosin-Phloxine Stain Set, Sol'n B: Phloxine B Stock Stain 1%, Aqueous
Part Number: 1082
CAS-No.: Not applicable
SDS Number: 3820

1.2 Recommended Use: Laboratory Chemicals

1.3 Company: Newcomer Supply
 2505 Parview Road
 Middleton, WI 53562 USA

Telephone: 1-800-383-7799

Fax: 1-608-831-0866

Website: www.newcomersupply.com

Email: newly@newcomersupply.com

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 leak, fire, exposure or other accident.

2. HAZARD(S) IDENTIFICATION

2.1 Classification of the substance or mixture
 GHS Classification, (in accordance with 29 CFR1910.1200)
 None

2.2 GHS Label elements
Signal Word NONE

Pictogram
Hazard Statement(s):

· None

Precautionary Statement(s):

· None

2.3 Description of any hazards not otherwise classified None

2.4 >1% of mixture with unknown acute toxicity None

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.2 Mixture
Hazardous Components

Component		Concentration
Name	Phloxine B	
CAS-No.	18472-87-2	1%

4. FIRST-AID MEASURES
4.1 Description of necessary measures
Inhalation (breathing)

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing. Get medical advice/attention if you feel unwell.

Skin Contact

Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. Wash contaminated clothing before reuse. If skin irritation occurs: Get medical advice/attention.

Eye Contact

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. If eye irritation persists get medical advice/attention.

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Ingestion (swallowed)

IF SWALLOWED: Rinse mouth. Do NOT induce vomiting. Call a POISON CENTER or doctor/physician if you feel unwell.

4.2 Most important symptoms and or effects, acute and delayed

The most important symptoms/effects are presented in Section 2 and or Section 11.

4.3 Indication of any immediate medical attention and special treatment needed

No data available

5. FIRE-FIGHTING MEASURES
5.1 Suitable extinguishing media

Carbon dioxide, dry chemical, water spray, alcohol-resistant foam.

5.2 Specific hazards arising from the substance or mixture

No data available

5.3 Protective equipment and precautions for fire-fighters

Wear a positive-pressure self-contained breathing apparatus if necessary. Wear chemical resistant clothing as recommended by clothing manufacturer.

NFPA Rating

Health	Fire	Reactivity
hazard: 0	hazard: 0	hazard: 0

6. ACCIDENTAL RELEASE MEASURES
6.1 Personal precautions, protective equipment and emergency procedures

Apply personal protective equipment (see Section 8). Use in a properly ventilated area. Avoid breathing vapors. Avoid skin contact. Avoid eye contact. Wash hands after use.

6.2 Methods and material for containment and cleaning up

Apply personal protective equipment (see Section 8). Contain spill. Prevent further leakage if possible and safe to do so. Ensure proper ventilation. For small amounts, wipe or absorb spill using inert material and dispose of according to local regulations. For large amounts, evacuate area and limit access. Prevent entry of material into sewage drains and confined areas. Dispose of any contaminated materials according to local regulations.

7. HANDLING AND STORAGE
7.1 Precautions for safe handling

Do not breathe dust/fume/gas/mist/vapours/spray. Do not get in eyes, on skin, or on clothing.

7.2 Conditions for safe storage, including any incompatibilities

Refer to Section 2.2 for proper storage temperature. Store the tightly closed container in a cool, dry, well-ventilated area.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION
8.1 Control Parameters

Does not contain components with occupational exposure limits.

8.2 Exposure Controls
Appropriate engineering controls

Use in a properly ventilated area. Remove/wash before reuse contaminated clothing. Wash hands upon exiting work premises.

8.3 Personal Protective Equipment
Eye/Face protection

Version 1.0

Wear chemical safety goggles and/or a full face shield if splashing is possible. Keep eye wash fountain nearby.

Skin Protection

Wear chemical-resistant gloves. Gloves should be resistant to components of product. Refer to glove manufacturer for appropriate type and glove thickness.

Body Protection

No data available

Respiratory Protection

No data available

Other Information

None

9. PHYSICAL AND CHEMICAL PROPERTIES
9.1 Information on basic physical and chemical properties

Physical state	Red liquid
Odor	Odorless
Odor threshold	No data available
pH	No data available
Melting point/freezing point	ca. 0°C (ca. 32°F)
Initial boiling point and boiling range	ca. 100°C (ca. 32°F)
Flash point	No data available
Evaporation rate	Evap. rate of water = 1; 1
Flammability (solid, gas)	No data available
Upper flammability or explosive limits	No data available
Lower flammability or explosive limits	No data available
Vapor pressure	18 mm Hg at 20°C
Vapor density	For water in air = 1; 1
Relative density	Similar to water
Solubility(ies)	Water soluble
Partition coefficient: n-octanol/water	No data available
Auto-ignition temperature	No data available
Decomposition temperature	No data available
Viscosity	1.222 (mPa)(s) at 20°C

10. STABILITY AND REACTIVITY
10.1 Reactivity

No data available

10.2 Chemical stability

Stable in a closed container within label-specified storage temperature and expiration date.

10.3 Possibility of hazardous reactions

No data available

10.4 Conditions to avoid

Avoid heat, spark, and flames

10.5 Incompatible materials

Strong oxidizing agents and strong bases

10.6 Hazardous decomposition products

No data available

11. TOXICOLOGICAL INFORMATION

Version 1.0

11.1 Information on toxicological effects**Inhalation exposure**

No data available

Oral exposure

No data available

Dermal exposure

No data available

Skin corrosion/irritation

No data available

Serious eye damage/irritation

No data available

Respiratory or skin sensitization

No data available

Germ Cell mutagenicity

No data available

Reproductive toxicity

No data available

Specific target organ toxicity - single exposure

No data available

Specific target organ toxicity - repeated exposure

No data available

Aspiration hazard

No data available

Acute toxicity

No data available

Carcinogenicity

IARC: None of the components are listed

IARC: None of the components are listed

IARC: None of the components are listed

Additional information

RTECS: No data available

12. ECOLOGICAL INFORMATION**12.1 Ecotoxicity**

No data available

12.2 Persistence and degradability

No data available

12.3 Bioaccumulative potential

No data available

12.4 Mobility in soil

No data available

12.5 Other adverse effects

No data available

Version 1.0

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Contaminated packaging

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UN-Number	No data available
Proper shipping name	No data available
Hazard class	No data available
Packing group	No data available
Environmental hazards	No data available

15. REGULATORY INFORMATION

15.1 No data available

16. OTHER INFORMATION

Preparation Information
Newcomer Supply Inc.
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www.newcomersupply.com
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