

## Thermo Scientific Shandon Staining Products Instructions for Use

Thermo Scientific Shandon Staining Products provide a total solution to your staining needs.

Shandon stains are intended for use in histological and cytological staining procedures. All are formulated and packaged to provide our customers with the best value. Every reagent and raw material is tested to meet the highest standards. Only those dyes certified by the Biological Stain Commission are used in manufacturing. Each lot of stain is subjected to a series of quantitative and qualitative tests. These tests ensure maximum performance, stability and lot to lot consistency. From scientific testing and spectrophotometry, to staining a simple control sample, we make every effort to guarantee your satisfaction with Shandon stains and reagents.

**Gill Hematoxylin**s offer speed, convenience and the choice of stain intensity. Gill 1 is recommended for cytology preparations. For darker nuclei or shorter staining times Gill 2 may be used. Gill 2 or Gill 3 is recommended for routine histology, immunocytochemistry and frozen section applications. Gill 3 is also recommended for the staining of resin embedded tissue sections.

**Harris Hematoxylin**s are ready-to-use in your choice of acidified or non-acidified formulations. The Thermo Scientific brand does not employ a mercury-based oxidizing agent, thus removing a possible source of environmental contamination.

**Bluing Reagent** is a gentle, pH-controlled solution for bluing hematoxylin. It replaces the strong alkaline rinses or long tap water washes which may cause loss of cells or tissue sections.

**Nu-Clear™** improves cell clarity by removing excess hematoxylin from the tissue in regressive staining protocols. It may also be used to remove background hematoxylin from the slide. Nu-Clear is designed for hand staining as well as use on automatic stainers. It is available in 2 concentrations: Nu-Clear I is faster acting, with a 10-second differentiating step; Nu-Clear II will accomplish similar results in 20 seconds. The less concentrated Nu-Clear II is recommended for use on Linear stainers and with procedures that use Gill Hematoxylin. The usage and timing depend on the staining intensity preferred by the laboratory.

**Cytoplasmic Counterstains** are offered in a complete range for use with routine H & E or Papanicolaou techniques. The range includes Instant Eosin and alcoholic or aqueous Eosin Y for Histology, as well as EA-50, EA-65 and OG-6 for Cytology. All stains produce crisp, brilliant staining to compliment the nucleus and differentiate various cellular components.

**Xylene Substitute** is an odorless Xylene Substitute. A safe and convenient alternative to xylene it is an aliphatic hydrocarbon that is not greasy. It may be used on stainers, automatic coverslippers and tissue processors.

### General Comments

- Shandon stains are designed to be used full strength.
- If less intense cytoplasmic staining is desired, Eosin-Y can be diluted with 80-85% alcohol (ethanol reagent or alcohol).
- Harris Hematoxylin and Gill 3 should be filtered before use.
- The coating agent found in Thermo Scientific Shandon Cell-Fixx and Thermo Scientific Shandon Cytospin Collection Fluid must be removed before staining. Generally, soaking in 95% alcohol will remove the coating. Failure to completely remove the coating will result in poor and uneven staining. Users of commercial spray fixatives should follow the manufacturer's recommendation for removal.
- If using a mercuric chloride fixative (i.e. B-5, Zenkers) for histology specimens remove the mercuric chloride crystals with iodine and clear with sodium thiosulfate after removing the wax and before the hematoxylin step.
- The pH of tap water varies considerably. Use of tap water in the staining procedures may give unpredictable results. Long-running tap water rinses are not recommended. Chlorine in tap water will bleach out the stain.
- Following are guidelines for cytology and histology staining procedures. Suggested staining times and number of rinses can be adjusted to suit individual preferences in stain intensity.

**NOTE:** The following protocols have not been validated and no responsibility is taken for their use. Customers should always validate protocols before placing any reliance on them.

### Storage

Store away from direct sunlight and at room temperature. Do not freeze. Do not handle or store near heat, sparks, flames, or strong oxidants.

### Histology Staining Guidelines

Step	Solution	Time
1	Xylene Substitute or Xylene	3-4 minutes
2	Xylene Substitute or Xylene	3-4 minutes
3	Xylene Substitute or Xylene	3-4 minutes
4	Alcohol, 100%	1 minute
5	Alcohol, 95%	1 minute
6	Alcohol (optional)	1 minute
7	Distilled Water Wash	1-2 minutes
8	Harris Hematoxylin (filter before use)	4-8 minutes
	OR Gill Hematoxylin (depending on formulation 2 or 3)	2-5 minutes
9	Distilled Water Wash	1-2 minutes
10	Nu-Clear I	10 seconds
	OR Nu-Clear II	20 seconds
11	Distilled Water Wash	1 minute
12	Bluing Reagent	1 minute
13	Distilled Water Wash	1-2 minutes
14	Alcohol (70%-95% optional)	1 minute
15	Eosin Y, alcoholic	10 seconds to 1 minute
	OR Eosin, aqueous	2-4 minutes
16	Alcohol, 95%	20-30 seconds
17	Absolute Alcohol	1-2 minutes
18	Absolute Alcohol	1-2 minutes
19	Absolute Alcohol	1-2 minutes
20	Xylene Substitute or Xylene	1-2 minutes
21	Xylene Substitute or Xylene	1-2 minutes
22	Xylene Substitute or Xylene	1-2 minutes
23	Mount with Histo-Mount or Shandon-Mount	

### Cytology Staining Guidelines

Step	Solution	Time
1	95% Alcohol (if needed)	10-15 minute pre-soak
	Distilled Water Wash	1 minute
2	Harris Hematoxylin (filtered immediately before use)	3 minutes
	OR Gill Hematoxylin (depending on formulation 1, 2 or 3)	1 to 4 minutes
3	Distilled Water Wash	30-60 seconds
4	Nu-Clear I	10 seconds
	OR Nu-Clear II	20 seconds
5	Distilled Water Wash	30-60 seconds
6	Bluing Reagent	1 minute
7	Distilled Water Wash	30-60 seconds
8	95% Alcohol	10 seconds
9	Orange G-6	1-3 minutes
10	95% Alcohol	10 seconds
11	95% Alcohol	10 seconds
12	EA-50	3 to 6 minutes
	OR EA-65	3 to 6 minutes
13	95% Alcohol	10 seconds
14	95% Alcohol	10 seconds
15	Absolute Alcohol	20 seconds
16	Absolute Alcohol	20 seconds
17	Xylene Substitute or Xylene	1 minute
18	Xylene Substitute or Xylene	1 minute
19	Xylene Substitute or Xylene	5 minutes
20	Mount with Histo-Mount or Shandon-Mount	

### Warnings and Precautions

FOR IN VITRO DIAGNOSTIC USE. Reasonable care should be taken when using all laboratory reagents. Use only with adequate ventilation. Keep away from direct sunlight. Contains FLAMMABLE liquids.

See Material Safety Data Sheets for warnings and precautions, as well as R & S code definitions.

See container labels for warnings and precautions.

**Order Information****Counterstains**

<b>Product</b>	<b>Size</b>	<b>Unit</b>	<b>REF</b>
Eosin-Y (Alcoholic)	1L (0.26 gal.)	2/cs.	6766007
	4L (1.06 gal.)	ea.	6766008
Eosin (Aqueous)	1L (0.26 gal.)	2/cs.	6766009
	4L (1.06 gal.)	ea.	6766010
Orange G-6	1L (0.26 gal.)	2/cs.	6766005
	4L (1.06 gal.)	ea.	6766006
EA-50	1L (0.26 gal.)	2/cs.	6766001
	4L (1.06 gal.)	ea.	6766002
EA-65	1L (0.26 gal.)	2/cs.	6766003
	4L (1.06 gal.)	ea.	6766004
Bluing Reagent	1L (0.26 gal.)	2/cs.	6769001
	4L (1.06 gal.)	ea.	6769002
Nu-Clear I	4L (1.06 gal.)	ea.	6769008
Nu-Clear II	4L (1.06 gal.)	ea.	6769009
Xylene Substitute	4L (1.06 gal.)	ea.	9990505

**Hematoxylin**

<b>Product</b>	<b>Size</b>	<b>Unit</b>	<b>REF</b>
Harris (Acidified)	1L (0.26 gal.)	2/cs.	6765003
	4L (1.06 gal.)	ea.	6765004
Harris (Non-acidified)	1L (0.26 gal.)	2/cs.	6765001
	4L (1.06 gal.)	ea.	6765002
Gill 1	1L (0.26 gal.)	2/cs.	6765005
	4L (1.06 gal.)	ea.	6765006
Gill 2	1L (0.26 gal.)	2/cs.	6765007
	4L (1.06 gal.)	ea.	6765008
Gill 3	1L (0.26 gal.)	2/cs.	6765009
	4L (1.06 gal.)	ea.	6765010

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Anatomical Pathology



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