epredia Enhancing precision cancer diagnostics

Instant Hematoxylin, Instant Eosin Alcoholic and Instant Eosin Aqueous

Series S

Instructions for Use

Intended Use

For in vitro diagnostic use.
Instant Hematoxylin is intended to be used as a nuclear stain for the diagnosis of general pathology specimens.

Instant Eosin Alcoholic and Instant Eosin Aqueous are each intended to be used as a cytoplasmic stain for the diagnosis of general pathology specimens.

Introduction

Epredia™ Instant Hematoxylin and instant eosins are intended for use in histological and cytological staining procedures. All are formulated and tested to meet the highest quality standards, only using dyes certified by the Biological Stain Commission. Each lot of stain undergoes qualitative testing, ensuring optimal performance and lot-to-lot consistency.

Instant Hematoxylin

Instant Hematoxylin is an innovative concept in nuclear staining and can be substituted for Gill, Harris, or Mayer hematoxylins. By altering the amount of water used during preparation, Instant Hematoxylin is suitable for both histology and cytology staining, including immunoperoxidase techniques. Instant Hematoxylin does not stain mucin and may be used progressively or regressively. Timing will be longer for regressive procedures.

Note: In cytology staining, Instant Hematoxylin step time will depend on the amount of water used for reconstitution (see "Mixing Instructions").

Shelf Life
Instant Hematoxylin has a 48 month shelf-life in the dry state. Once it has been reconstituted, it is good for approximately 6 months if properly stored.

Storage
Instant Hematoxylin (both dry and reconstituted) should be stored at room temperature in tightly closed containers and kept away from direct sunlight.

Mixing Instructions
1. Pour entire contents of one "Part A" and one "Part B" into a container that will hold over 1 liter of solution.
2. Add 1.0 liter of deionized or distilled water. DO NOT USE TAP WATER.
3. Stir or shake the solution for several minutes or until powders are dissolved. Wait at least 1 hour before using. Best results are obtained after 12 hours.
4. Filter before using. A precipitate and surface scum are normal for this product.
5. A more delicate stain may be desired for cytology procedures. In that case, the amount of water used in mixing can be increased from 1.0 liter to 1.5 or 2.0 liters.

Instant Eosin Alcoholic

Instant Eosin Alcoholic is an innovative concept in cytoplasmic staining for histology and can be directly substituted for any alcoholic eosin Y counterstain. If staining is too dark, the stain may be diluted 1:1 with 70% ethanol or denatured ethanol. Alternatively, the dry powder stain may be reconstituted with 2 liters of solvent. The formation of a pink precipitate is normal for this product and may be filtered out with no adverse effect on staining.

Shelf Life
Instant Hematoxylin has a 48 month shelf-life in the dry state. Once the stain has been reconstituted, the stain is good for approximately 2 months if properly stored.

Storage
Instant Eosin Alcoholic (both dry and reconstituted) should be stored at room temperature in tightly closed containers and kept away from direct sunlight.

Mixing Instructions
1. Fill flask or other suitable container with 1.0 liter of 70% ethanol or denatured ethanol. To make a 70% solution, combine 700 mL absolute alcohol with 300 mL deionized or distilled water.

   Note: This is an alcoholic eosin formulation. Do not reconstitute using only water.
2. Carefully pour contents of one Instant Eosin bottle into the alcohol solution.
3. Mix for 15 – 60 minutes until all components have dissolved. Time required is dependent upon temperature.

   Warning: DUE TO THE FLAMMABILITY OF THE SOLVENT, DO NOT HEAT THE SOLUTION TO HASTEN RECONSTITUTION.
4. Use immediately or store in a tightly capped container.

Instant Eosin Aqueous

Instant Eosin Aqueous is an innovative concept in cytoplasmic staining for histology and can be directly substituted for any aqueous eosin Y counterstain.

Shelf Life
Instant Hematoxylin has a 48 month shelf-life in the dry state. Once the stain has been reconstituted, the stain is good for approximately 2 months if properly stored.

Storage
Instant Eosin Aqueous (both dry and reconstituted) should be stored at room temperature in tightly closed containers and kept away from direct sunlight.

Mixing Instructions
1. Fill a suitable container with 1.0 liter deionized or distilled water.
2. Carefully pour contents of Instant Eosin Aqueous bottle into the water.
3. Mix for 1 – 2 minutes until contents have dissolved. Time required is dependent upon temperature.
4. Use immediately or store in a tightly capped container.

Bluing Reagent

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™

Nu-Clear improves cellular clarity by removing excess hematoxylin from the tissue, as well as background hematoxylin from the slide. Nu-Clear is designed for both manual and automated staining. It is available in two concentrations: Nu-Clear I is faster acting, with a 10-second differentiating step; Nu-Clear II will accomplish similar results in 20 seconds. Nu-Clear I is recommended for use with Harris hematoxylin, whereas Nu-Clear II is recommended for use with Gill hematoxylin. The overall usage and final timing of Nu-Clear depend on the staining intensity preferred by the laboratory.

Cytoplasmic Counterstains

Cytoplasmic counterstains are used within routine H&E or Papanicolaou techniques and produce bright, well-differentiated staining to distinguish the nucleus and various cellular components. These stains include alcoholic and aqueous eosin formulations for histology, as well as Orange G-6, EA-50, and EA-65 for cytology.

General Comments

1. If using a mercuric chloride fixative (e.g. B-5, Zenker's) for histology specimens, utilize an iodine-sodium thiosulfate sequence to remove the mercury pigment after deparaffinization of the sections and prior to hematoxylin staining.
2. If using Cell-Fixx™ or Cytospin™ Collection Fluid, the coating agent must be removed before staining. Generally, soaking in 95% Dehydrant for 15 minutes 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.
3. The pH of tap water varies considerably. Use of tap water in the staining procedures may produce variable staining results. Long-running tap water rinses are not recommended. Chlorine in tap water will bleach out the stain.
4. The alcohol used in the staining procedure may be either reagent alcohol or ethanol.
5. It is recommended that control slides be run at a frequency established by the laboratory to verify the quality of the staining process.
6. The following are guidelines for histology and cytology staining procedures. Suggested staining times and number of rinses can be adjusted to suit individual preferences in stain intensity.

   NOTE: All results should be tested and validated following normal laboratory procedure.

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Histology Staining Guidelines

Instant Hematoxylin and Instant Eosin Alcoholic

Step	Solution	Time
1	Xylene or Xylene Substitute	3 minutes
2	Xylene or Xylene Substitute	3 minutes
3	Xylene or Xylene Substitute	3 minutes
4	100% Dehydrant	1 minute
5	100% Dehydrant	1 minute
6	100% Dehydrant	1 minute
7	95% Dehydrant	1 minute
8	Rinse in running tap water	Briefly
9	Deionized or distilled water	Rinse
10	Instant Hematoxylin (filter before use) (depending on strength)	2 – 5 minutes
11	Deionized or distilled water	Rinse off excess stain (agitate)
12	Nu-Clear I	10 seconds
	OR Nu-Clear II	20 seconds
13	Deionized or distilled water	1 minute
14	Bluing Reagent	1 minute
15	Deionized or distilled water	1 minute (agitate)
16	95% Dehydrant	30 seconds
17	Instant Eosin Alcoholic	30 seconds to 2 minutes
18	95% Dehydrant	20 seconds to 1 minute
19	95% Dehydrant	20 seconds to 1 minute
20	100% Dehydrant	1 minute
21	100% Dehydrant	1 minute
22	100% Dehydrant	1 minute
23	Xylene or Xylene Substitute	1 minute
24	Xylene or Xylene Substitute	1 minute
25	Xylene or Xylene Substitute	1 minute

Instant Hematoxylin and Instant Eosin Aqueous

Step	Solution	Time
1	Xylene or Xylene Substitute	3 minutes
2	Xylene or Xylene Substitute	3 minutes
3	Xylene or Xylene Substitute	3 minutes
4	100% Dehydrant	1 minute
5	100% Dehydrant	1 minute
6	100% Dehydrant	1 minute
7	95% Dehydrant	1 minute
8	Rinse in running tap water	Briefly
9	Deionized or distilled water	Rinse
10	Instant Hematoxylin (filter before use) (depending on strength)	2 – 5 minutes
11	Deionized or distilled water	Rinse off excess stain (agitate)
12	Nu-Clear I	10 seconds
	OR Nu-Clear II	20 seconds
13	Deionized or distilled water	1 minute (agitate)
14	Bluing Reagent	1 minute
15	Deionized or distilled water	1 minute (agitate)
16	Instant Eosin Aqueous	3-4 minutes
17	Deionized or distilled water	30 seconds
18	95% Dehydrant	1 minute
19	95% Dehydrant	1 minute
20	100% Dehydrant	1 minute
21	100% Dehydrant	1 minute
22	100% Dehydrant	1 minute
23	Xylene or Xylene Substitute	1 minute
24	Xylene or Xylene Substitute	1 minute
25	Xylene or Xylene Substitute	1 minute

Cytology Staining Guidelines

Instant Hematoxylin, Orange G-6, and EA-50/EA-65

Step	Solution	Time
	95% Dehydrant (if needed)	10 – 15 minute pre-soak
1	Deionized or distilled water	1 minute
2	Instant Hematoxylin (filter before use) (depending on strength)	1-4 minutes
3	Deionized or distilled water	30 – 60 seconds (agitate)
4	Nu-Clear I	10 seconds
	OR Nu-Clear II	20 seconds
5	Deionized or distilled water	30 – 60 seconds (agitate)
6	Bluing Reagent	1 minute
7	Deionized or distilled water	30 – 60 seconds (agitate)
8	95% Dehydrant	30 seconds
9	Orange G-6	1 – 3 minutes
10	95% Dehydrant	30 seconds
11	95% Dehydrant	30 seconds
12	EA-50	3 to 6 minutes
	OR EA-65	3 to 6 minutes
13	95% Dehydrant	30 seconds
14	95% Dehydrant	30 seconds
15	100% Dehydrant	1 minute
16	100% Dehydrant	1 minute
17	100% Dehydrant	1 minute
18	Xylene or Xylene Substitute	1 minute
19	Xylene or Xylene Substitute	1 minute
20	Xylene or Xylene Substitute	1 minute

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	Size	Qty.	REF
Instant Hematoxylin (makes 1 L x 6)		6 sets/cs.	6765015
Instant Eosin Alcoholic (makes 1 L x 6)		6/cs.	6765040
Instant Eosin Aqueous (makes 1 L x 6)		6/cs.	6765540
Bluing Reagent (Series S)	1 L (0.26 gal.)	2/cs.	6769001
Bluing Reagent (Series S)	1 L (0.26 gal.)	4/cs.	6769002
Nu-Clear™ I	3.8 L (1 gal.)	Ea.	6769008
Nu-Clear™ II	3.8 L (1 gal.)	Ea.	6769009
Orange G-6 (Series S)	1 L (0.26 gal.)	2/cs.	6766005
Orange G-6 (Series S)	3.79 L (1.0 gal.)	Ea.	6766006
EA-50 (Series S)	1 L (0.26 gal.)	2/cs.	6766001
EA-50 (Series S)	3.79 L (1.0 gal.)	Ea.	6766002
EA-65 (Series S)	1 L (0.26 gal.)	2/cs.	6766003
EA-65 (Series S)	3.79 L (1.0 gal.)	Ea.	6766004

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