HARRIS HEMATOXYLIN & EOSIN STAIN

DESCRIPTION

Harris Hematoxylin is used for histology specimen staining. This stain can be used with regressive or progressive stain methods offering a dark blue nuclear stain. Use this stain if the Gill 3X Hematoxylin staining is not providing a dark enough nuclear stain. Alternatively, use the Harris, Acidified as a progressive method for fewer steps and the same good quality.

PROTOCOL FOR HARRIS H&E STAIN FOR HISTOLOGY

1. **Paraffin slides:** Deparaffinize in xylene, then rehydrate through graded alcohols to water.
   Frozen slides: Fix in 95% Ethanol (or other fixative according to lab preference) and rinse in water.
2. Stain in Hematoxylin Solution, Harris (filter before use): 15 minutes for paraffin slides, 1 to 2 minutes for frozen sections
3. Rinse in two changes of DI water: 30 seconds each
   Note: this could also be done using running tap water, but we recommend DI water to avoid the varying pH found in tap water
4. Differentiate *in* Acid Alcohol 0.5%, or Acid Alcohol 1%: 10 seconds
   Note: Some prefer to use a weaker acid alcohol rinse which is 0.5% while others prefer the 1% acid alcohol rinse. The higher percent solution will extract dye a little fast but there is not much difference from the weaker solution here. SKIP THIS STEP FOR FROZEN SECTIONS.
5. Wash in running tap water for 2 minutes
6. Bluing Reagent (until tissue turns blue): 30 to 60 seconds
7. Rinse in running tap water for 2 minutes
8. Ethanol 95%: 15 to 30 seconds
9. Eosin Y 1% Alcoholic, or Eosin Y Intensified, or Eosin-Phloxine: 1 to 2 minutes
   Note: For an increased definition of cytoplasmic components over Eosin Y 1%, select Eosin Y Intensified or Eosin-Phloxine as an alternative.
10. Dehydrate in Ethanol 95%, two changes: 10 dips each
11. Dehydrate in Ethanol 100%, two changes: 1 minute each
12. Clear in Xylene or Xylene substitute, two changes: 1 minute each

PROTOCOL FOR HARRIS HEMATOXYLIN, ACIDIFIED & EOSIN STAIN FOR HISTOLOGY (PROGRESSIVE PROCEDURE)

1. **Paraffin slides:** Deparaffinize in xylene, then rehydrate through graded alcohol to water.
   Frozen slides: Fix in Ethanol 95% (or other fixative according to lab preference) and rinse in water.
2. Stain in Hematoxylin solution, Harris Hematoxylin, acidified (filter before use): 5 to 10 minutes. 2 to 4 minutes for frozen section slides
3. Rinse in two changes of DI water: 30 seconds each
   Note: This could also be done using running tap water, but we recommend DI water to avoid the varying pH found in tap water.
4. Bluing Reagent (until tissue turns blue): 15 to 60 seconds
5. Rinse in running tap water for 2 minutes
6. Ethanol 95%: 15 to 30 seconds
7. Eosin Y 1% Alcoholic, Eosin Y Intensified, or Eosin-Phloxine: 1 to 2 minutes
   Note: for an increased definition of cytoplasmic components over Eosin Y 1%, select Eosin Y Intensified or Eosin-Phloxine as a substitute.
8. Dehydrate in Ethanol 95%, two changes: 10 dips each
9. Dehydrate in Ethanol 100%, two changes: 1 minute each
10. Clear in Xylene or Xylene Substitute, two changes: 1 minute each.
11. Coverslip using Acrylic Mounting Medium and examine under microscope.

RESULTS

Nuclei, nuclear components - blue to dark purple
Cytoplasm, red blood cells, collagen, elastic fiber, muscle- shades of pink

ORDERING INFORMATION

Cat. #	Description
3315	Deionized Water
7000	Hematoxylin Harris, Hg Free
7002	Hematoxylin Harris, Hg Free, Acidified
7560	Acid Alcohol, 0.5%
7561	Acid Alcohol 1%
3556	Bluing Reagent
7006	Eosin Y, 1% Alcoholic
7007	Eosin Y, Intensified
7009	Eosin- Phloxine
3340	Ethanol Solution, 95%
3341	Ethanol Solution, 100%
3346	Xylene
3349	Acrylic Mounting Medium

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