

INTENDED USE

EZ Carmine (dye) is used to stain acidic mucopolysaccharides.

PRODUCT SUMMARY

Carminic acid is an extract of cochineal insects. Carminic acid when combined with a positively charged mordant (aluminum) forms the carmine dye. The cationic carmine dye binds to the anionic mucin. Neither DNA nor RNA will stain with the carmine dye presumably because of the large size of the carmine molecule (steric hindrance). Carmine dye is used to prepare the standard mucin staining solution, Mucicarmine.

INGREDIENTS

Carmine (C.I. 75470)

WARNING

Irritant to eyes.

For In Vitro Diagnostic Use.

STORAGE

Store at room temperature, away from direct sunlight. Keep containers tightly closed when not in use.

DIRECTIONS FOR USE

1. EZ Mucicarmine stain preparation (Anatech Ltd formulation for mucicarmine)

- a. Carmine dye solution
EZ Carmine..... 5.0 g
Deionized or distilled water..... 250 ml
- b. Aluminum chloride..... 5.0 g
Quickly add to carmine dye solution.

Caution: addition of aluminum chloride generates a significant amount of HCl gas. Perform this step under an exhaust hood. Wear gloves, goggles, and apron.

- c. Add 750 ml deionized water.
 - d. Add 0.01 g thymol crystals.
 - e. Mix 1 hour on a magnetic stirrer.
 - f. Let stand overnight.
 - g. Filter before use.
 - h. Store EZ Mucicarmine solution in the refrigerator.
2. Staining
- a. EZ Mucicarmine stain stains acidic mucins pink to red, depending upon their degree of acidity. Sulfated mucosubstances stain more intensely than carboxylated mucins. Neutral mucins do not stain.
 - b. A compatible counterstain for EZ Mucicarmine is EZ Green (ANATECH Catalog #873). Collagen and muscle will stain green. RNA rich cells retain the hematoxylin, thus cytoplasm may appear bluish-green. Exposure to acid alcohol usually reduces this blue cast depending on RNA content.

RECOMMENDED STAINING SCHEDULE

- 1. Clearant x 3 3 minutes each
- 2. 100% alcohol x 2 1 minute each
- 3. 95% alcohol 1 minute
- 4. 50% alcohol 1 minute
- 5. Distilled or deionized water x 2 30 seconds each
- 6. Harris Hematoxylin variable
- 7. Distilled or deionized water rinse
- 8. Acid alcohol variable
- 9. Tap water rinse
- 10. Bluing reagent variable
- 11. Tap water rinse
- 12. EZ Mucicarmine 10 minutes
- 13. Distilled or deionized water rinse
- 14. EZ Green or appropriate counterstain
- 15. Distilled or deionized water 1 minute
- 16. 70% alcohol 1 minute
- 17. 95% alcohol 1 minute
- 18. 100% alcohol x 3 1 minute each
- 19. Clearant x 3 1 minute each

Results: Acidic mucin–pink to red; Nuclei–blue; Collagen, cytoplasm and muscle–dependent on counterstain

DISPOSAL

- 1. Use a licensed waste hauler.
- 2. Discard into the sanitary sewer system with the approval of local wastewater officials.

MSDS

MSDS are available online at www.anatechltusa.com.

ORDERING INFORMATION FOR EZ CARMINE

<u>Cat#</u>	<u>Packaging</u>
872	EZ Carmine, 25 g jar
878	EZ Carmine, 1 kg
871	EZ Mucicarmine, 1 quart
884	Mucicarmine Stain Kit