Indigo Carmine (Indigotine, E 132)

Catalogue No. ASD-639

1. Identification

Chemical name	Disodium 3,3′-dioxo-2,2′-bi-indolylidene-5,5′-disulfonate
Synonyms	Indigotine; Indigo-5,5′-disulfonic acid disodium salt; Acid Blue 74; CI Food Blue 1; FD&C Blue No. 2
C.I. number	73015
E number	E 132
CAS number	860-22-0
EC number	212-728-8
Molecular formula	$C_{16}H_8N_2Na_2O_8S_2$
Molecular weight	466.35 g/mol
Dye class	Indigoid, anionic (acid) dye

2. Intended Use

Histological counterstain, redox indicator, alkaline pH indicator, teaching reagent and E 132 reference colour.

For laboratory, research, education and quality-control use. Not intended for human or veterinary therapeutic use, diagnosis, or direct consumption. Food-grade (E 132) material. For food-industry supply, contact Ausamics for suitability and regulatory documentation.

3. Mode of Action

The two sulfonate groups make the dye anionic, so it binds positively charged tissue proteins such as collagen in acidic stains. The indigoid chromophore is reversibly reduced to a yellow leuco form, and above about pH 12 the dye changes from blue to yellow.

4. Physical & Chemical Properties

Appearance	Dark blue powder
Colour of aqueous solution	Blue
Absorption maximum (λmax)	approx. 610 nm in water

Solubility	Water: approx. 10 g/L at 25 °C; slightly soluble in ethanol
pH indicator range	pH 11.6 (blue) to 14.0 (yellow)
Redox behaviour	Reduced to a yellow leuco form; re-oxidised to blue by air
Light sensitivity	Light sensitive; protect solutions and powder from light

5. Specifications

Parameter	Specification
Physical appearance	Blue powder
Total dye content (dried basis, 105 ± 1 °C, 2 h)	Min. 85 %
Loss on drying at 135 °C, plus chlorides and sulfates (as sodium salts)	Max. 15 %
Water-insoluble matter	Max. 0.2 %
Combined ether extracts	Max. 0.2 %
Subsidiary dyes	Max. 1 %
Isatin sulfonic acid	Max. 0.5 %
Lead (Pb)	Max. 2 ppm
Arsenic (As)	Max. 3 ppm
Heavy metals	Max. 40 ppm

6. Solution Preparation

1. Stock solution (1 % w/v): weigh 1.0 g of indigo carmine.
2. Add to about 80 mL of purified water and stir, warming gently to 40–50 °C if needed.
3. Make up to 100 mL with purified water and filter if required.
4. Store in an amber bottle at room temperature. Dilute for use according to your method.

7. Applications

- Histology: Counterstain in picro-indigo carmine methods, giving blue-green collagen against red or magenta nuclei.
- Redox Chemistry: Redox indicator: blue when oxidised, yellow in the reduced leuco form. Used in oxygen-sensitive reactions.

- Alkaline pH Indicator: Colour change from blue to yellow between pH 11.6 and 14.0 in strongly alkaline solutions.
- Education: Classic glucose–indigo carmine “traffic light” demonstration of oxidation and reduction.
- Food Colour QC: Reference material for E 132 identification, spectrophotometry and colour-matching work.
- Microscopy & Tracing: Water-soluble blue dye for contrast, tracing and visualisation in research models.

8. Storage & Shelf Life

Store at 15–25 °C, dry, tightly closed and protected from light. Use before the expiry date on the label. Prepare working solutions fresh when colour intensity is critical.

9. Literature / References

5. Commission Regulation (EU) No 231/2012, Annex: E 132 Indigotine, Indigo carmine. Specifications for food additives.
6. FAO/WHO JECFA. Indigotine: Combined Compendium of Food Additive Specifications, Monograph 1.
7. EFSA ANS Panel (2014). Scientific Opinion on the re-evaluation of indigo carmine (E 132) as a food additive. EFSA Journal 12(7):3768.
8. Horobin R.W., Kiernan J.A. (eds) (2002). Conn's Biological Stains, 10th ed. BIOS Scientific Publishers, Oxford.
9. Suvarna S.K., Layton C., Bancroft J.D. (eds) (2019). Bancroft's Theory and Practice of Histological Techniques, 8th ed. Elsevier.

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