

Product Data Sheet

Product Name: Ferrozine

Cat. No.: GC47346

Chemical Properties

Cas. No. 69898-45-9

SMILES OS(C1=CC=C(C2=C(C3=CC=C(S([O-])(=O)=O)C=C3)N=C(C4=CC=CC=N4)N=N2)C=C1)(=O)=O.[Na+]

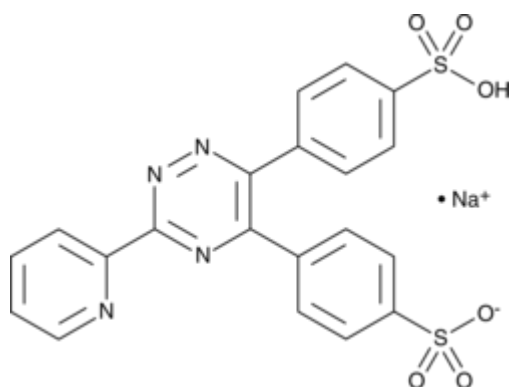
Formula $C_{20}H_{13}N_4O_6S_2.Na$ M.Wt 492.5

Solubility PBS (pH 7.2): 1 mg/ml Storage Store at -20°C

General For obtaining a higher solubility, please warm the tube at 37 °C and shake it in the tips ultrasonic bath for a while. Stock solution can be stored below -20°C for several months.

Shipping Evaluation sample solution : ship with blue ice All other available size: ship with RT, or Condition blue ice upon request.

Structure



Protocol

This plan only provides a guide, please modify it to meet your specific needs.

1. Divide the equally divided sample of cell lysate (100µL) Placed in Eppendorf tube and connected to 100µL 10mM HCl (Fe^{3+} solvent) and 100µL iron release reagent (dissolved in an equal volume of 1.4M HCl and 4.5% (w/v) $KMnO_4$ in ultrapure water, freshly prepared) mixed;

Note: $HCl/KMnO_4$ pretreatment can quantitatively release iron from proteins, including iron storage protein ferritin and heme proteins such as hemoglobin. The digestion of iron containing proteins mediated by $HCl/KMnO_4$ is essential for the iron quantification of heme proteins. For example, if the

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protein is not pretreated with an acidic permanganate solution, treating hemoglobin with iron zinc will not result in the recovery of any Fe^{2+} - Ferrozine complexes.

2. Incubate the mixture in a fume hood at 60°C for 2 hours, as chlorine gas will be generated during the reaction process;
3. After cooling the mixture to room temperature, add 30µL of iron detection reagents (6.5mM Ferrozine, 6.5mM neocuproine, 2.5M ammonium acetate, and 1M ascorbic acid dissolved in water) to each tube;
4. After 30 minutes, remove 280µL from each tube the solution into the well of a 96 well plate and measure the absorbance at 562nm on a microplate reader.
5. 100µL FeCl_3 standard (0-300µM) in 10 mM HCl, 100µL 50 mM NaOH, 100µL release reagent and 30µL mixture in the detection reagent. Calculate the iron content of the sample by comparing its absorbance with that of an equal volume standard concentration range prepared in a similar manner to the sample.

Notes:

- 1) Fluorescent dyes all have quenching problems, please try to avoid light as much as possible to slow down fluorescence quenching.
- 2) For your safety and health, please wear laboratory clothes and disposable gloves when operating.

References:

[1]. Jan Riemer, et al. Colorimetric ferrozine based assessment for the quantification of iron in cultured cells 2004 Aug 15; 331 (2): 370-5. doi: 10.1016/j.ab. 2004.03.049.

Background

Ferrozine reacts with divalent iron to form a stable magenta complex species and used for the direct determination of iron in water with maximum absorbance at 562 nm [1,2]. The visible absorption spectrum of the ferrous complex of ferrozine exhibits a single sharp peak at 562 nm. At this wavelength, the molar absorptivity is 27,900 and the Beer-ambert law is obeyed to approximately 4 mg/L of Fe [1].

Ferrozine assay of ISE6 cells[3]

The ferrozine assay for measuring non-haem iron was adapted to determine the concentration of iron in ISE6 cells. After knockdown and/or iron exposure, cells were collected, and cell lysates were collected using the method described above. Concentrated HCl (99.5%) was added and then heated to 95 °C. After cooling to room temperature, the mixture was centrifuged, and the supernatant was

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obtained, to which was added 75 mM ascorbate or water. Afterward, 10 mM ferrozine was added. Saturated ammonium acetate was added to facilitate colour development. Absorbance was measured at 550 nm, and the iron concentration was calculated based on a molar extinction coefficient of the iron-ferrozine complex of 27,900 M⁻¹cm⁻¹ and based on the protein concentration. The protein concentration was measured using a Micro BCA Protein Assay Kit. The total iron concentration is computed from samples with ascorbate. The ferrous iron concentration was computed from samples without ascorbate (reducing agent), while the ferric iron concentration is computed from the difference between the total iron and ferrous iron concentration.

References:

- [1]. Stookey L L. Ferrozine---a new spectrophotometric reagent for iron[J]. Analytical chemistry, 1970, 42(7): 779-781.
- [2]. Jeitner T M. Optimized ferrozine-based assay for dissolved iron[J]. Analytical biochemistry, 2014, 454: 36-37.
- [3]. Hernandez E P, Kusakisako K, Talactac M R, et al. Induction of intracellular ferritin expression in embryo-derived Ixodes scapularis cell line (ISE6)[J]. Scientific reports, 2018, 8(1): 1-12.

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