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Chrome Azural S (CAS) Plate Assay for Iron-Binding Compounds

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Protocol status: Working

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Abstract

Please contact Dr. Steven Wilhelm (wilhelm@utk.edu) for additional information regarding this protocol.

Modified from Schwyn, B. & Neilands, J. B. Universal chemical assay for the detection and determination of siderophores. *Anal Biochem*, 160:147-156 (1987).

Materials

STEP MATERIALS

 Piperazine anhydrous

 Iron(III) chloride hexahydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944

 CTAB (Hexadecyltrimethylamm onium bromide) BBI Biotech Catalog #CB0108-100g

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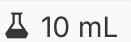
 Iron(III) chloride hexahydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944

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Assay Solution Preparation

- 1 Take 6 mL 10 mM CTAB (HDTMA) stock and mix with 40 mL Milli-Q H₂O
 CTAB (Hexadecyltrimethylammonium bromide) BBI
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- 2 Mix 1.5 mL FeCl₃-HCl stock (1 mM FeCl₃ dissolved in 10 mM HCl) with 7.5 mL 2 mM CAS dye stock
 Iron(III) chloride hexahydrate Merck MilliporeSigma (Sigma-Aldrich) Catalog #44944
- 3 Slowly add Fe-CAS mixture to CTAB solution while stirring
- 4 Add 6.5 mL of 12 N HCl slowly to 25 mL Milli-Q H₂O
- 5 Add 4.3 g anhydrous piperazine to the acid solution
 4.3 g
 Piperazine anhydrous
- 6 Mix the piperazine acid solution slowly into the Fe-CAS*CTAB solution
- 7 Bring CAS solution to 100 mL final volume
 100 mL
- 8 Add enough 5-sulfosalicylic acid to get 4 mM final concentration

Assay Part I

- 9 Add 60.5 mg of 2 mM CAS dye to 50 mL Milli-Q H₂O
- 10 Add 10 mL FeCl₃ solution
 10 mL
- 11 Dissolve 73 mg of 10 mM CTAB (HDTMA) in 40 mL Milli-Q H₂O

- 12 Mix the two solutions together
- 13 Bring solution to 1 L with Milli-Q H₂O

- 14 Autoclave at 121°C for 20 min

Assay Part II

- 15 Make medium depending on the type of microorganism that you are trying to grow.

Note

For example, add ESAW-FE with casamino acids, peptone and agar for marine microorganisms.
- 16 Autoclave medium at 121°C for 20 min
- 17 Once both part I and part II are cooled (55°C), mix the two solutions together and pour plates.
- 18 Incubate plates according to the microorganism. Screen plates once colonies are grown: yellow halos = siderophore and blue = no siderophore