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

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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
- 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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Assay Solution Preparation

1 Take 6 mL 10 mM CTAB (HDTMA) stock and mix with 40 mL Milli-Q H_2O

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

2 Mix 1.5 mL $FeCl_3$-HCl stock (1 mM $FeCl_3$ 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 H_2O

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-sulfosalicyclic 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 H_2O

10 Add 10 mL $FeCl_3$ solution

10 mL

11 Dissolve 73 mg of 10 mM CTAB (HDTMA) in 40 mL Milli-Q H_2O

12 Mix the two solutions together

13 Bring solution to 1 L with Milli-Q H_2O

1 L

14 Autoclave at 121ºC 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 121ºC for 20 min

17 Once both part I and part II are cooled (55ºC), 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