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

Dr. Steven Wilhelm

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).

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Protocol

Assay Solution Preparation

Step 1.

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

REAGENTS
CTAB (Hexadecyltrimethylamm onium bromide) CB0108-100g by BBI Biotech

Step 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

REAGENTS
Iron(III) chloride hexahydrate 44944 by Sigma Aldrich

Step 3.

Slowly add Fe-CAS mixture to CTAB solution while stirring

Step 4.

Add 6.5 mL of 12 N HCl slowly to 25 mL Milli-Q H_2O

Step 5.

Add 4.3 g anhydrous piperazine to the acid solution

AMOUNT
4.3 g Additional info:

REAGENTS
Piperazine anhydrous by Contributed by users

Step 6.

Mix the piperazine acid solution slowly into the Fe-CAS*CTAB solution

Step 7.

Bring CAS solution to 100 mL final volume

AMOUNT
100 ml Additional info:

Step 8.

Add enough 5-sulfosalicyclic acid to get 4 mM final concentration

Assay Part I

Step 9.

Add 60.5 mg of 2 mM CAS dye to 50 mL Milli-Q H_2O

Step 10.

Add 10 mL $FeCl_3$ solution

AMOUNT
10 ml Additional info:

Step 11.

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

Step 12.

Mix the two solutions together

Step 13.

Bring solution to 1 L with Milli-Q H_2O

AMOUNT
1 L Additional info:

Step 14.

Autoclave at 121ºC for 20 min

Assay Part II

Step 15.

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

NOTES
Alyssa Alsante 09 Jun 2017

For example, add ESAW-FE with casamino acids, peptone and agar for marine microorganisms.

Step 16.

Autoclave medium at 121ºC for 20 min

Step 17.

Once both part I and part II are cooled (55ºC), mix the two solutions together and pour plates.

Step 18.

Incubate plates according to the microorganism. Screen plates once colonies are grown: yellow halos = siderophore and blue = no siderophore