Chrome Azurol S (CAS) Liquid Assay for Iron-Binding Compounds Version 2

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

Assay Solution Preparation

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

Assay Solution Preparation

Step 3.

Slowly add Fe-CAS mixture to CTAB solution while stirring

Assay Solution Preparation

Step 4.

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

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Assay Solution Preparation

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

Assay Solution Preparation

Step 6.

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

Assay Solution Preparation

Step 7.

Bring CAS solution to 100 mL final volume

AMOUNT

100 ml Additional info:

Assay Solution Preparation

Step 8.

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

CAS Assay

Step 9.

Take a sample to test and dilute it up to 0.5 mL final volume with Chelex-100 treated water

AMOUNT

0.5 ml Additional info:

REAGENTS

Chelex-100 resin by Contributed by users

CAS Assay

Step 10.

Add 0.5 mL CAS solution

AMOUNT

0.5 ml Additional info:

CAS Assay

Step 11.

Let sit 1 hr at room temperature in the dark

DURATION

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01:00:00

CAS Assay

Step 12.

For controls, your blank is 0.5 mL chelex-100 treated water. A series of dilutions of EDTA can be used for a standard curve.

CAS Assay

Step 13.

To measure the results, place the sample in a spectrophotometer and read the absorbance at 630 nm

NOTES

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The absorbance will decrease as more siderophore is present

CAS Assay

Step 14.

To graph/report the result, you calculate the absorbance of the blank/absorbance of the sample

NOTES

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If no chelators are present, the number will = 1.0, and if something binds iron the number will increase.

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