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# 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<sub>2</sub>O



REAGENTS

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

#### **Assay Solution Preparation**

### Step 2.

Mix 1.5 mL FeCl<sub>3</sub>-HCl stock (1 mM FeCl<sub>3</sub> 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**

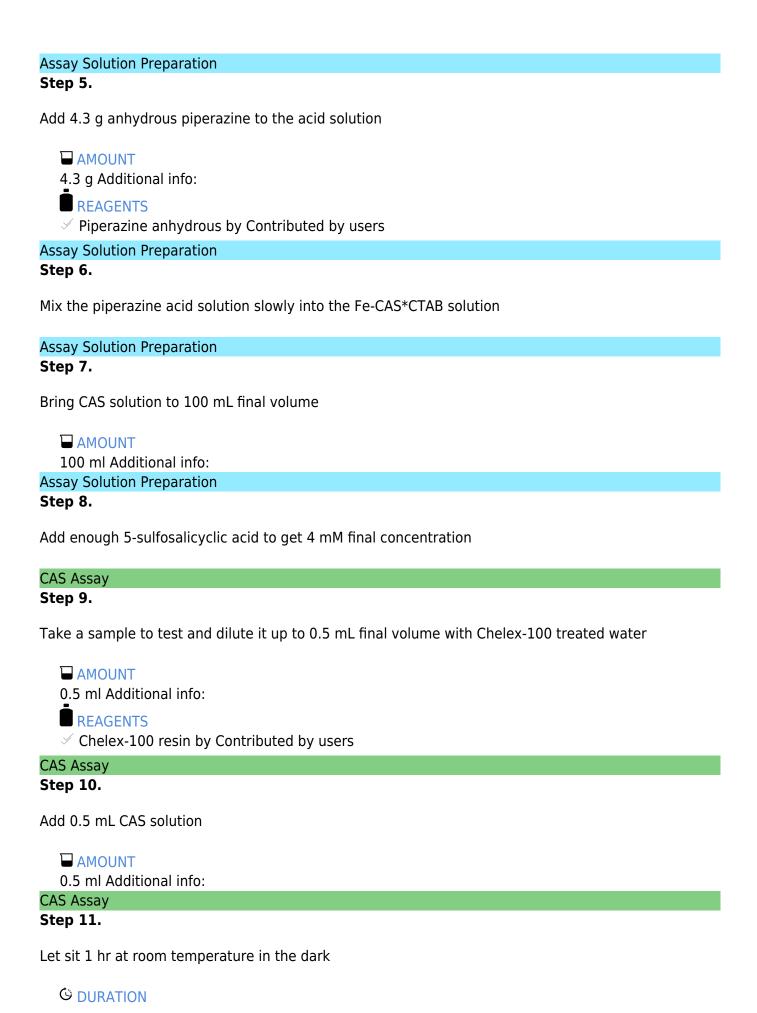
## Step 3.

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<sub>2</sub>O



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 Assav

## **Step 13.**

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

#### **P** NOTES

Alyssa Alsante 07 Jun 2017

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

## **P** NOTES

## Alyssa Alsante 07 Jun 2017

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