Published: 21 Jun 2017

Czaky Test for Hydroxamate-Type Siderophores

Dr. Steven Wilhelm

Abstract

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

Modified from Csaky T. Z. (1948) On the estimation of bound hydroxylamine in biological materials. Acta Chem. Scand. **2**, 250-454

and A. H. Gillam, A. G. Lewis, and R. J. Andersen. Quantitative determination of hydroxamic acids Anal. Chem., 1981, 53 (6), pp 841–844 DOI: 10.1021/ac00229a023

Citation: Dr. Steven Wilhelm Czaky Test for Hydroxamate-Type Siderophores. protocols.io

dx.doi.org/10.17504/protocols.io.icqcavw

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Protocol

Step 1.

Bring a siderophore sample to 2 mL in a 20 mL tube

■ AMOUNT

2 ml Additional info:

Step 2.

Add 2 mL 3 M sulfuric acid and autoclave for 4 hr at 121°C

■ AMOUNT

2 ml Additional info:

REAGENTS

Sulfuric acid A300C-212 by Fisher Scientific

© DURATION

04:00:00

NOTES

Alyssa Alsante 07 Jun 2017

Use H₂O instead of sulfuric acid for a blank sample.

Step 3.

AMOUNT 7 ml Additional info:
REAGENTS Sodium acetate <u>View</u> by <u>P212121</u>
Step 4.
Add 2 mL 1% sulfanilamide (in 30% v/v acetic acid) and mix
AMOUNT 2 ml Additional info:
■ REAGENTS ✓ Sulfanilamide by Contributed by users
Step 5.
Add 2 mL 0.65% iodine (in 1% w/v potassium idoide) and mix
AMOUNT 2 ml Additional info:
REAGENTS lodine View by P212121
Step 6.
Let sit for 5 min
© DURATION 00:05:00
Step 7.
Add 2 mL 1.5% sodium arsenate and mix
AMOUNT 2 ml Additional info: Step 8.
Add 2 mL 0.05% n-(napthyl) ethylene diamine (NNED) and mix
AMOUNT 2 ml Additional info: Step 9.
Let sit for 30 min
© DURATION 00:30:00 Step 10.

After cooling, add 7 mL 2 M sodium acetate and mix

Spin out particulate matter

Step 11.

Read at 543 nm using a spectrophotometer

Step 12.

For a standard curve, use hydroxylamine

