MA-0170

Nickel Colorimetric Assay

(100 wells, Colorimetric, OD 330 nm & 405 nm, Store at room temperature)

Introduction:

Nickel is one of the most common of the metallic elements in the earth's crust, 2nd only to iron. A number of enzymes utilize nickel as part of the active site, as a mononuclear (glyoxalase I, superoxide dismutase); dinuclear (urease); or more complex heteronuclear cluster (several hydrogenases and dehydrogenases). We take advantage of Nickel's ability to form complexes with sulfhydryl compounds which possess absorbance bands in the UV/visible region. AkrivisBio's Nickel Assay is a simple, sensitive way to quantify Ni^{2+} in a wide range of sample types. Since other ions also exhibit similar behavior with sulfur-containing chemicals, a differential absorbance method is used to correct for interfering substances.

Assay Principle:

In borate buffer, Nickel forms a complex with mercaptoethanol, giving a strong absorbance band at 330 nm
If iron and/or cobalt are present, they also show differential absorbance at 330 and 405 nm which are used to correct for those influences. Magnesium, copper and zinc do not interfere.

Assay Components:

Assay Buffer	20 ml	WM	MA-0170A
Mercaptoethanol	1 ml	Green	MA-0170B
Nickel Chloride	lyoph	Yellow	MA-0170C

Storage and Handling:

Assay Buffer and Mercaptoethanol: Ready to use as supplied. Store at room temperature.
Nickel Standard: Dissolve in 1 ml DI H_2O to make a 1 mM solution. Store at room temperature.

Assay Protocol:

1. **Standard Curve:** Add 0, 10, 20, 30, 40, 50 µl of Nickel standard to a series of wells of a 96 well plate. Adjust all well volumes to 200 µl with Assay Buffer. The standards are 0 – 10 – 20 – 30 – 40 – 50 nmol Nickel.
2. **Sample Preparation:** Nickel can potentially vary over a broad range of concentrations. Add 10-100 µl of each sample and adjust all test well volumes to 200 µl with Assay Buffer. Sample readings should be within the range of the standard curve. If any samples exceed that range, dilute appropriately and rerun.

Note: If there is no or minimal iron or cobalt contamination, it's only necessary to read absorbance at 405 nm. If iron or cobalt are expected to be present in samples, read the samples at both 330 nm and 405 nm both before and after addition of mercaptoethanol.

3. **Reading 1:** Read OD of samples and standards at 330 nm and 405 nm before adding mercaptoethanol. Any absorbance is due only to Fe^{2+} and/or reagent background. Refer to these measurements as $OD330_1$ and $OD405_1$.
4. **Complex Development:** Add 10 µl of mercaptoethanol to all standard and sample wells and allow 30 minutes for complex formation and color development.
5. **Reading 2:** Read OD of all samples and standards again at 330 nm and 405 nm. Refer to these measurements as $OD330_2$ and $OD405_2$.
6. **Nickel Determination in the absence of iron or cobalt ions**: Subtract reading 1 at 405 nm ($OD405_1$) from reading 2 at 405 nm ($OD405_2$). Plot the standard curve. Determine the slope of the standard curve (OD/nmol). Divide the ΔOD405 ($OD405_2$-$OD405_1$) for the samples by the slope of the standard curve to determine nmoles of Nickel.
7. **Nickel Determination in the presence of Iron and/or Cobalt**: Subtract the 0 Nickel OD reading from all standard and sample readings.

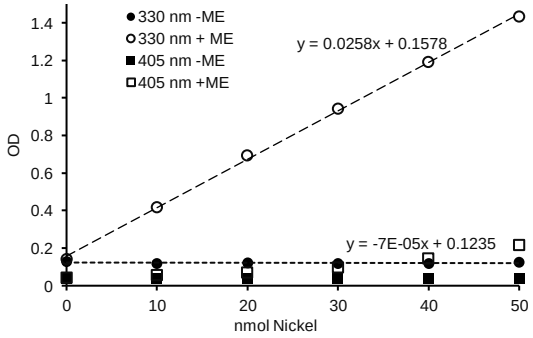
   a. Correction for interference at 330 nm due to Iron: In the absence of mercaptoethanol, absorbance at 330 nm ($OD330_1$) is contributed only by Fe^{2+}. After adding mercaptoethanol, Fe^{2+} contribution to the $OD330_2$ is determined as follows: **Calculated $OD330_2$ = 1.82 X $OD330_1$**. Subtract calculated $OD330_2$ value due to Iron from total measured $OD330_2$, to get corrected OD330 due to Nickel and Cobalt.

   b. Correction for interference at 405 nm due to Fe: In the absence of mercaptoethanol, absorbance at 405 nm ($OD405_1$) is contributed only by Iron. After adding mercaptoethanol, Iron contribution to $OD405_2$ can be calculated as follows: **Calculated $OD405_2$ = 1.65 X $OD405_1$**. Subtract the calculated $OD405_2$ value due to Iron from the total measured $OD405_2$ reading, to get the corrected OD405 reading due to only Nickel and Cobalt.

   c. Correction for interference due to Cobalt*: Calculate the ratio of corrected OD330 and OD405 = (**Corrected OD330/ Corrected OD405)**. The ratio will vary from 0.925 for 100% Cobalt contribution to absorbance to 2.8125 for 100% Nickel contribution. Subtract 0.925 from the ratio of corrected readings and divide that result by 1.8875, {[(OD330/OD405) – 0.925)]/1.8875}. This is the percentage of absorbance due to Nickel. Multiply the percentage by the corrected OD330 to get isolated Nickel absorbance at $OD330_2$, in samples.

Typical Results:

nmol	330 nm		405 nm	
Standard	- ME	+ OD	- ME	+
0	0.1276	0.1406	0.0388	0.0413
10	0.1191	0.4175	0.0383	0.0544
20	0.1227	0.6927	0.0381	0.0689
30	0.1194	0.9417	0.0385	0.0981
40	0.1182	1.1888	0.039	0.1432
50	0.1242	1.4325	0.0404	0.2165



8. **Calculation:** Plot the standard curve (ΔOD405, or ΔOD330). Determine the slope of the standard curve. Calculate the sample Nickel readings ΔOD405 from step 6 for samples without Iron or cobalt or corrected OD330 from step 7 for samples with Iron and/or Cobalt interference. Divide the corrected sample nickel readings by the slope of the Standard Curve to determine nmoles of Nickel in the well.

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