Nickel Assay Kit

(Catalog #K510-100; 100 assays; Store at room temperature)

I. Introduction:

Nickel is one of four ferromagnetic elements (symbol Ni, at. Num. 28). Several enzymes depend on nickel for activity, including some ureases, carbon monoxide dehydrogenases (methane forming enzymes which reduce CO_2 to CH_4) and some hydrogenases which allow the production or removal of H_2. Most of these activities are found in the archaebacteria. Nickel forms complexes with sulfhydryl compounds with significant absorbance in the UV/visible region in the presence of other ions. BioVision's Nickel Assay kit provides a simple method of quantifying Ni^{2+} in a variety of samples. The assay takes advantage of reaction of Ni^{2+} with mercaptoethanol in borate buffer to form a complex with strong absorbance bands from ~300 to 600 nm. Fe^{2+} and Co^{2+} interfere with the assay, therefore extra steps (as described below) must be taken to subtract the interference in order to determine the correct Nickel concentration in mixed samples. Other ions tested (Mn^{2+}, Cu^{2+}, Zn^{2+}) do not interfere with the assay, presumably no other ionic species are present in high enough concentration to interfere with the reaction. The assay is a simple method of quantifying Ni^{2+} in a variety of samples, which gives a linear range of 2 to 50 nmol Nickel containing less than 25 nmol Cobalt.

II. Kit Contents:

Components	K510-100	Cap Code	Part Number
Nickel Assay Buffer	20 ml	WM	K510 -100-1
Nickel Reagent	1 ml	Green	K510 -100-2
Nickel Chloride Standard (1.0 µmol)	lyophilized	Yellow	K510 -100-3

III. Reagent Preparation and Storage Conditions:

Store kit at room temperature, keep tightly capped.

Nickel Assay Buffer and Nickel Reagent: Ready to use as supplied. Store at room temperature. Stable for at least 6 months.

Nickel Standard: Dissolve in 1 ml dH_2O to make a 1 mM solution. Store at room temperature.

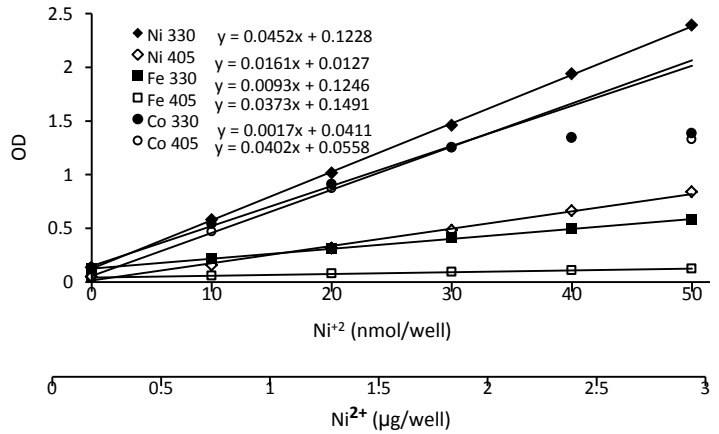
IV. Nickel Assay Protocol:

1. **Standard Curve Preparations:** Add 0, 10, 20, 30, 40, 50 µl of the Nickel standard to a series of wells. Adjust volume to 200 µl/well with Nickel Assay Buffer to generate 0, 10, 20, 30, 40 and 50 nmol per well of the Nickel Standard.
2. **Sample Preparation:** Sample Nickel concentrations can vary over a wide range. Take samples between 10-100 µl and adjust volume to 200 µl with Nickel Assay Buffer for each well. For unknown samples, different sample amounts should be tested to ensure the readings are within the standard curve linear range.

   Note: In the absence of Fe^{2+} and Co^{2+} in samples, the protocol requires reading OD405 nm only. In the presence of Fe^{2+} and Co^{2+} in samples, the protocol requires two separate readings at two different wavelengths to correct interference from Fe^{2+} and/or Co^{2+}.
3. **Reading 1:** Read OD of the samples and standards at 330 nm and 405 nm before adding the Nickel Reagent. This OD is due only to $Fe^{2+}$and reagent background. Call these measurements $OD330_1$ and $OD405_1$.
4. **Development:** Add 10 µl of Nickel Reagent to all standard and sample wells. Incubate at room temperature for 30 min to form complex.
5. **Reading 2:** Read OD at 330 nm and 405 nm. Call these measurements $OD330_2$ and $OD405_2$.
6. **Nickel Determination in the absence of Fe^{2+}, or/and Co^{2+}**: Subtract reading 1 ($OD405_1$) from reading 2 ($OD405_2$) to get the corrected reading ΔOD405. Plot the standard curve. Apply corrected ΔOD405 of unknown samples to the standard curve to determine Ni^{2+} amount in the reaction wells (Ay). Calculate Nickel concentration as in step 8 (without the step 7 corrections).
7. **Nickel Determination in the presence of Fe^{2+} or/and Co^{2+}**: Subtract the 0 Nickel OD reading from all standard, sample readings to correct absorbance due to buffer and plate.
   1) **Remove interference at 330 nm due to Fe^{2+}:** In the absence of Nickel Reagent, $OD330_1$ is contributed only by Fe^{2+}. After adding Nickel Reagent, Fe^{2+} contribution to the $OD330_2$ can be calculated as follows: **$FeOD330_2 = 1.82 \times OD330_1$**. Subtract the $FeOD330_2$ value from total $OD330_2$, to get corrected OD330, $\Delta FeOD330_2 = OD330_2 - FeOD330_2$, which is contributed by Ni^{2+} and Co^{2+}.
   2) **Remove interference at 405 nm due to Fe:** In the absence of Nickel Reagent $OD405_1$ is contributed only by Fe^{2+}. After adding Nickel Reagent, Fe^{2+} contribution to $OD405_2$ can be calculated as follows: **$FeOD405_2 = 1.65 \times OD405_1$**. Subtract $FeOD405_2$ value from total $OD405_2$ reading, to get corrected $OD405_2$ reading, $\Delta FeOD405_2 = OD405_2 - FeOD405_2$, which is contributed by only Ni^{2+} and Co^{2+}.
   3) **Remove interference due to Co^{2+}:** Calculate the ratio of $\Delta FeOD330_2$ and $\Delta FeOD405_2$: **$\Delta FeOD330_2/\Delta FeOD405_2$**. The ratio should fall between 0.925 (100% Co) and 2.8125 (100% Ni). Subtract 0.925 from the ratio calculated and divide that result by 1.8875, $(\Delta FeOD330_2/\Delta FeOD405_2 - 0.925)/1.8875$ is the percentage of absorbance due to Ni^{2+}. Multiply that percentage by $\Delta FeOD330_2$ to get Nickel absorbance at $OD330_2$, $\Delta FeCoOD330_2$ in samples.
8. **Calculation:** Plot the standard curve (ΔOD405, or ΔOD330). Calculate sample Nickel reading ΔOD405 from step 6 for samples without Fe^{2+} and Co^{2+}, or $\Delta FeCoOD330_2$ from step 7 for samples with Fe^{2+} or/and Co^{2+} interference. Apply the sample readings to the standard curve to get Ni^{2+} amounts (Ay) in the reaction well.

   Nickel Concentration = Ay/Sv (nmol/ml)

   Where: Ay is the amount of Ni^{2+} (nmol) in your sample from the standard curve.
   Sv is the sample volume (in ml) added to the sample well.
   Nickel molecular weight: 58.7 g/mol.



Nickel Standard Curve: Assays were performed following the kit protocol.

RELATED PRODUCTS:

Cobalt Assay Kit	Chloride Assay Kit
Iron Assay Kit	Phosphate Assay Kit
Calcium Assay Kit	Magnesium Assay Kit
Ammonia Assay Kit	Urea Assay Kit
Nitric Oxide Assay Kits	Glutathione Assay Kit

FOR RESEARCH USE ONLY! Not to be used on humans.

GENERAL TROUBLESHOOTING GUIDE:

Problems	Cause	Solution
Assay not working	• Use of ice-cold assay buffer	• Assay buffer must be at room temperature
	• Omission of a step in the protocol	• Refer and follow the data sheet precisely
	• Plate read at incorrect wavelength	• Check the wavelength in the data sheet and the filter settings of the instrument
	• Use of a different 96-well plate	• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates
Samples with erratic readings	• Use of an incompatible sample type	• Refer data sheet for details about incompatible samples
	• Samples prepared in a different buffer	• Use the assay buffer provided in the kit or refer data sheet for instructions
	• Samples were not deproteinized (if indicated in datasheet)	• Use the 10 kDa spin cut-off filter or PCA precipitation as indicated
	• Cell/ tissue samples were not completely homogenized	• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope
	• Samples used after multiple free-thaw cycles	• Aliquot and freeze samples if needed to use multiple times
	• Presence of interfering substance in the sample (e.g. metal ion chelators)	• Troubleshoot if needed, deproteinize samples
	• Use of old or inappropriately stored samples	• Use fresh samples or store at correct temperatures till use
Lower/ Higher readings in Samples and Standards	• Improperly thawed components	• Thaw all components completely and mix gently before use
	• Use of expired kit or improperly stored reagents	• Always check the expiry date and store the components appropriately
	• Allowing the reagents to sit for extended times on ice	• Always thaw and prepare fresh reaction mix before use
	• Incorrect incubation times or temperatures	• Refer datasheet & verify correct incubation times and temperatures
	• Incorrect volumes used	• Use calibrated pipettes and aliquot correctly
Readings do not follow a linear pattern for Standard curve	• Use of partially thawed components	• Thaw and resuspend all components before preparing the reaction mix
	• Pipetting errors in the standard	• Avoid pipetting small volumes
	• Pipetting errors in the reaction mix	• Prepare a master reaction mix whenever possible
	• Air bubbles formed in well	• Pipette gently against the wall of the tubes
	• Standard stock is at an incorrect concentration	• Always refer the dilutions in the data sheet
	• Calculation errors	• Recheck calculations after referring the data sheet; Correct for Fe^{2+} and Co^{2+}
	• Substituting reagents from older kits/ lots	• Use fresh components from the same kit
Unanticipated results	• Measured at incorrect wavelength	• Check the equipment and the filter setting
	• Samples contain interfering substances	• Troubleshoot if it interferes with the kit
	• Use of incompatible sample type	• Refer data sheet to check if sample is compatible with the kit or optimization is needed
	• Sample readings above/below the linear range	• Concentrate/ Dilute sample so as to be in the linear range
Note: The most probable list of causes is under each problem section. Causes/ Solutions may overlap with other problems.		