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Product Information

Nickel Assay Kit

Catalog Number **MAK027**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Nickel acts as a cofactor for several enzymes, including some ureases, carbon monoxide dehydrogenases (methane forming enzymes which reduce CO_2 to CH_4), and some hydrogenases. Nickel forms complexes with sulfhydryl compounds with significant absorbance in the UV/visible region in the presence of other ions.

In the Nickel Assay kit, Ni^{2+} reacts with 2-mercapto-ethanol 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 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+}, and Zn^{2+}) do not interfere with the assay and presumably no other ionic species will be 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 2–50 nmole nickel in samples containing <25 nmole of Co^{2+} ions.

Components

The kit is sufficient for 100 assays in 96 well plates.

Nickel Assay Buffer Catalog Number MAK027A	20 mL
Nickel Reagent Catalog Number MAK027B	1 mL
Nickel Chloride Standard, 1.0 µmole Catalog Number MAK027C	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents.

Nickel Assay Buffer – Ready-to-use as supplied. Store at 2–8 °C and use within 6 months.

Nickel Reagent – Ready-to-use as supplied. Store at 2–8 °C and use within 6 months.

Nickel Chloride Standard – Reconstitute with 1 mL of water to generate a 1 mM NiCl stock solution. Mix well by pipetting, then aliquot and store at 2–8 °C. Use within 6 months of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at 2–8 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Nickel Standards for Colorimetric Detection
Add 0, 10, 20, 30, 40, and 50 μL of the 1 mM standard solution into a 96 well plate, generating 0 (blank), 10, 20, 30, 40, and 50 nmole/well standards. Add Nickel Assay Buffer to each well to bring the volume to 200 μL.

Sample Preparation
Aliquot 10–100 μL of sample to a clean tube and adjust volume to 200 μL with Nickel Assay Buffer.

Notes: For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

In the absence of Fe^{2+} and Co^{2+} in samples, the procedure requires reading absorbance at 405 nm. In the presence of Fe^{2+} and Co^{2+} in samples, the procedure requires two separate readings at two different wavelengths to correct for interference.

Assay Reaction
1. Measure the absorbance of the samples and standards at 330 nm and 405 nm before adding Nickel Reagent to determine the initial readings, $(A_{330})_{initial}$ and $(A_{405})_{initial}$.

2. Add 10 μL of the Nickel Reagent to each of the wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation. Incubate the plate at room temperature for 30 minutes to allow the complex to form.

3. Measure the absorbance at 330 nm and 405 nm to determine the second reading $(A_{330})_2$ and $(A_{405})_2$.

Results

1. Subtract the blank reading from all standard and sample readings to correct absorbance.

Nickel Determination in the absence of Fe^{2+} and/or Co^{2+}

Subtract the initial reading, $(A_{405})_{initial}$, from the second reading, $(A_{405})_2$, to obtain the corrected reading, ΔA_{405} Plot the standard curve and compare the corrected ΔA_{405} of unknown samples to the standard curve to determine the Ni^{2+} in the sample wells (S_a). Calculate nickel concentration as described under Calculations.

Nickel Determination in the presence of Fe^{2+} and/or Co^{2+}

1. Remove interference at 330 nm due to Fe^{2+}. In the absence of the Nickel Reagent, $(A_{330})_{initial}$ is only due to the presence of Fe^{2+}. After addition of Nickel Reagent, the contribution of Fe^{2+} to $(A_{330})_2$ is calculated as follows:

$$Fe(A_{330})_2 = 1.65 \times (A_{330})_{initial}$$

Subtract the $Fe(A_{330})_2$ value from $(A_{330})_2$ to obtain the corrected $(A_{330})_2$, reflective of Ni^{2+} and Co^{2+} contribution:

$$\Delta Fe(A_{330})_2 = (A_{330})_{initial} - Fe(A_{330})_2$$

2. Remove interference at 405 nm due to Fe^{2+}. In the absence of Nickel Reagent, $(A_{405})_{initial}$ is only due to the presence of Fe^{2+}. After addition of Nickel Reagent, the contribution of Fe^{2+} to $(A_{405})_2$ is calculated as follows:

$$Fe(A_{405})_2 = 1.82 \times (A_{405})_{initial}$$

Subtract the $Fe(A_{405})_2$ value from $(A_{405})_2$ to obtain the corrected $(A_{405})_2$, reflective of Ni^{2+} and Co^{2+} contribution:

$$\Delta Fe(A_{405})_2 = (A_{405})_{initial} - Fe(A_{405})_2$$

3. Remove interference due to Co^{2+}. Calculate the ratio of $\Delta Fe(A_{330})_2$ and $\Delta Fe(A_{405})_2$:

$$R = \Delta Fe(A_{330})_2 / \Delta Fe(A_{405})_2$$

The ratio should fall between 0.925 (100% Co^{2+}) and 2.8125 (100% Ni^{2+}). Use the following equation to determine the percentage of absorbance due to Ni^{2+} and the absorbance of nickel at 330 nm, $\Delta FeCo(A_{330})_2$:

$$\% \text{ Nickel} = \frac{R - 0.925}{1.8875}$$

$$\Delta FeCo(A_{330})_2 = \Delta Fe(A_{330})_2 \times \% \text{ Nickel}$$

Calculations

Plot the nickel standard curve using either A_{405} or A_{330}. Note: A new standard curve must be set up each time the assay is run.

The amount of nickel present in the samples may be determined from the standard curve. Using the corrected measurements, ΔA_{405} for samples in absence of Fe^{2+} and/or Co^{2+}, or $\Delta FeCo(A_{330})_2$ for samples in the presence of Fe^{2+} and/or Co^{2+}, determine the amount of nickel in the sample wells (S_a).

Concentration of Nickel

$$C = S_a / S_v$$

S_a = Amount of nickel in unknown sample (nmole)
S_v = Sample volume (mL) added into wells
C = concentration of nickel in sample

The molecular weight of nickel is 58.7 g/mole.

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Cold assay buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of improperly stored reagents	Check and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	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 plate well
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

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