Version 2a Last updated 6 June 2023

ab241008
Nickel Assay Kit

For the measurement of Ni^{2+} in a range of biological samples.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

Table of Contents

1. Overview

Nickel Assay Kit (ab241008) 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.

Prepare standards and samples in Nickel Assay Buffer (200 µL final volume).

⇩

Add Nickel Reagent (10 µL).

⇩

Incubate for 30 minutes at room temperature.

⇩

Read OD405 nm (samples without Fe^{2+} or Co^{2+}) or at both OD330 nm and OD405 nm (samples containing Fe^{2+} and/or Co^{2+}).

2. Materials Supplied and Storage

Store kit at room temperature immediately on receipt and check below for storage for individual components. Kit can be stored for 6 months from receipt, if components have not been reconstituted.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Nickel Assay Buffer	20 mL	RT	RT
Nickel Reagent	1 mL	RT	RT
Nickel Chloride Standard (1 µmol)	1 vial	RT	RT

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Microplate reader capable of measuring absorbance at OD 330 nm and 505 nm.
- 96 well plate with clear flat bottom

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

5.1 Nickel Assay Buffer

Ready to use as supplied.

5.2 Nickel Reagent

Ready to use as supplied.

5.3 Nickel Chloride Standard (1 µmol)

Dissolve in 1 mL dH_2O to make a 1 mM solution. Store at room temperature.

6. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
-

6.1 Add 0, 10, 20, 30, 40, and 50 µL of 1 mM Nickel Chloride Standard into a series of wells in a 96-well plate to generate 0, 10, 20, 30, 40 and 50 nmol of Nickel Chloride/well. Adjust the volume to 200 µL/well with Nickel Assay Buffer.

Standard #	Nickel Chloride Standard (µL)	Nickel Assay Buffer (µL)	Final volume standard in well (µL)	Nickel Chloride Standard in well (nmol/well)
1	0	200	200	0
2	10	190	200	10
3	20	180	200	20
4	30	170	200	30
5	40	160	200	40
6	50	150	200	50

7. Sample Preparation

General sample information:

We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

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.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

Δ Note: In the absence of Fe^{2+} and Co^{2+} in samples, the protocol requires reading OD405 nm only. However, 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+}.

8.1 Reading 1:

Read OD of the samples (200 µL, see Section 7) and standards (200 µL, see Section 6) 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 $OD405_1$.

8.2 Development:

Add 10 µL of Nickel Reagent to all standard and sample wells. Incubate at room temperature for 30 minutes to form complex.

8.3 Reading 2:

Read OD at 330 nm and 405 nm. Call these measurements $OD330_2$ and $OD405_2$.

9. Data Analysis

9.1 Nickel determination in the absence of Fe^{2+} and/or 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 9.3 (without the Step 9.2 corrections).

9.2 Nickel determination in the presence of Fe^{2+} and/or Co^{2+}.

Subtract the 0 Nickel OD reading from all standard and sample readings to correct for 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: $FeOD330_2 = 1.82 \text{ X } OD330_1$. 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: $FeOD405_2 = 1.65 \text{ X } OD405_1$. 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.

9.3 Calculation: Plot the standard curve (ΔOD405, or ΔOD330). Calculate sample Nickel reading ΔOD405 from Step 9.1 for samples without Fe^{2+} and Co^{2+}, or $\Delta FeCoOD330_2$ from Step 9.2

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.

$$\text{Nickel Concentration (nmol/mL)} = \frac{\text{Ay}}{\text{Sv}}$$

Where:

Ay = amount of Ni^{2+} in the sample calculated from standard curve (nmol).

Sv = sample volume added in the sample wells (mL).

10. Typical Data

Data provided **for demonstration purposes only.**

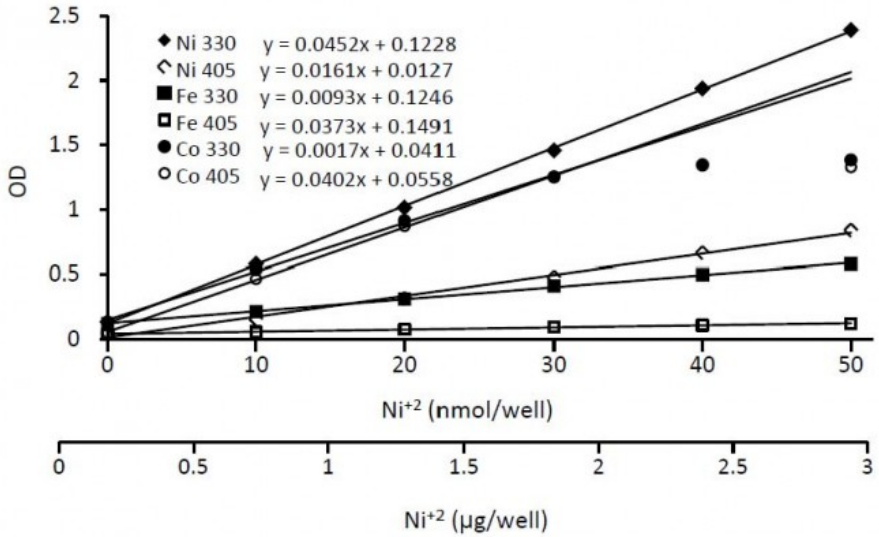

Figure 1. Nickel Standard Curve.

11. Notes

Technical Support

Austria
wissenschaftlicherdienst@abcam.com | 019-288-259
France
supportscientifique@abcam.com | 01.46.94.62.96
Germany
wissenschaftlicherdienst@abcam.com | 030-896-779-154
Spain
soportecientifico@abcam.com | 91-114-65-60

Switzerland
technical@abcam.com
Deutsch: 043-501-64-24 | Français: 061-500-05-30
UK, EU and ROW
technical@abcam.com | +44(0)1223-696000

Canada
ca.technical@abcam.com | 877-749-8807
US and Latin America
us.technical@abcam.com | 888-772-2226

Asia Pacific
hk.technical@abcam.com | (852) 2603-6823
China
cn.technical@abcam.com | +86-21-5110-5938 | 400-628-6880
Japan
technical@abcam.co.jp | +81-(0)3-6231-0940
Singapore
sg.technical@abcam.com | 800 188-5244

Australia
au.technical@abcam.com | +61-(0)3-8652-1450
New Zealand
nz.technical@abc.com | +64-(0)9-909-7829