ab285276 – Neomycin ELISA Kit

For for in vitro quantitative determination of Neomycin
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285276

Materials Supplied

Item	Quantity	Storage Condition
Micro ELISA Plate	8 wells ×12 strips	4°C
Standard (S0 – S6)	7 x 1 mL	4°C
Enzyme Conjugate	7 mL	4°C
Antibody Working Solution	7 mL	4°C
Substrate A	7 mL	4°C
Substrate B	7 mL	4°C
Stop Solution	7 mL	4°C
20X Wash Buffer	15 mL	4°C
Redissolving Solution (2X)	2 x 50 mL	4°C

Materials Required, Not Supplied

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

- Chemicals: 3% Trichloroacetic acid (TCA), 2% Trichloroacetic acid (TCA), NaOH
- Microplate reader capable of measuring absorbance at 450 nm
- Clean Eppendorf tubes & graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

Storage conditions

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

Reagent and Sample Preparation

Δ Note: *Bring all reagents to room temperature 30 minutes before use.*
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Redissolving Solution: mixed Redissolving Solution (2X) with deionized water at 1:1 (1 ml concentrated redissolving solution + 1 ml deionized water)

Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 40 ml of Wash Buffer (20X) into 760 ml deionized or distilled water to prepare 100 ml of Wash Buffer (1X). Keep it at 4°C for one month.

Standards Concentration: Ready to Use

Standards	S0	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1	1 ppb (High control)

Sample Preparation

Δ Note: *The prepared sample maybe stored for up to one day at 4°C.*

Tissue (Chicken/liver, pork/liver, shrimp, fish) sample
Take 2 g of the homogenized sample into 50 ml centrifuge tube, add 8 ml 3% Trichloroacetic acid, shake for 5 min, centrifuge at above 4000 r/min at room temperature (20-25°C) for 10 min. Take 2 ml supernatant to another tube, adjust PH to 7.0-7.5 with 2M NaOH (about 100 µl), Dilute it with diluted redissolving solution at 1:4 (100 µl sample + 400 µl diluted redissolving solution), mix for 30s. Take 50 µl for analysis. (Dilution factor: 20)

Honey, milk, milk powder sample
Take 2 g sample into 50 ml centrifuge tube, add 8mL 2% Trichloroacetic acid, shake for 5min, centrifuge at above 4000 r/min at room temperature (25°C) for 10 min. Take 2 ml supernatant to another tube, adjust pH to 8.0 with 2M NaOH (about 100 ul). Dilute it with diluted redissolving solution at 1:4 (100 µl sample + 400 µl diluted redissolving solution), mix for 30s. Take 50 µl for analysis. (Dilution factor: 20)

Assay Protocol

Δ Note: *Bring all reagents and samples to room temperature 30 minutes prior to the assay.*
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Prepare all reagents, samples and standards as instructed earlier.
2. Add 50 µl of the sample and standard solution to separate duplicate wells; Then 50 µl enzyme conjugate and 50 µl of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25°C for 30 min.
3. Wash the microplate with the washing buffer at 250 µl/well for 4-5 times. Each time soak the well with the washing buffer for 15-30 sec, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
4. Add 50 µl of the substrate A and then 50 µl of the substrate B into each well. Mix gently by shaking the plate manually, and incubate at 25°C for 15 min at dark for coloration.
5. Add 50 µl of the stop solution into each well. Mix gently by shaking the plate manually. Read the OD value at the dual-wavelength 450/630nm within 5 min

Calculation

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B0 X 100%

B: The average absorbance value of the sample or standard
B0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Fluoroquinolones standards solution (ppb) as x-axis. The Fluoroquinolones concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

Technical Support

For all technical or commercial enquiries please go to:

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