Biotin Quantification Kit

Catalog No: abx351107

Size: 96T

Range: 0.313 ng/ml - 20 ng/ml

Sensitivity: 0.188 ng/ml

Storage: Store the 96-well plate, Standards, and Detection Reagent(s) at -20°C, and the rest of the kit components at 4°C.

Application: The quantitative detection of Biotin.

Principle of the Assay: This kit is a competitive biochemical assay. Biotin is pre-coated onto a 96-well plate. Standards, samples, and Avidin-HRP are added to the wells and incubated. A competitive inhibition reaction takes place between the Biotin in the sample or standard, and the pre-coated Biotin, with the sites on the Avidin-HRP. Unbound conjugates are removed using wash buffer. TMB substrate is used to quantify the HRP enzymatic reaction. After TMB substrate is added, only wells that contain sufficient Biotin will produce a blue coloured product, which then changes to yellow after adding the acidic stop solution. The OD is measured spectrophotometrically at 450 nm in a microplate reader, from which the concentration of Biotin in samples can be calculated.

Kit Components

- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 2 tubes
- Sample/Standard Diluent Buffer: 20 ml
- Wash Buffer: (25X) 30 ml
- Avidin-HRP: (100X) 120 µl
- Avidin-HRP Diluent: 10 ml
- TMB Substrate: 10 ml
- Stop Solution: 10 ml
- Plate Sealer: 3
- Hermetic Bag: 1

Materials Required But Not Provided

- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- Microplate reader (wavelength: 450 nm)
- ELISA Shaker

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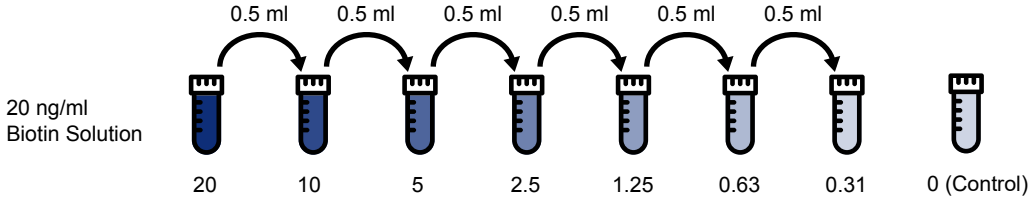
Protocol

A. Reagent Preparation

Standard: Centrifuge the standard at 10,000 x g for 1 min. Prepare the Standard with 1 ml of Standard Diluent buffer to make the 20 ng/ml Standard Solution. This is the highest standard. Allow the reconstituted standard to sit for 10 mins, with gentle agitation prior to carrying out the serial dilutions. Avoid foaming or bubbles. Label tubes in preparation for the serial dilutions - *see the diagram below for reference*. Aliquot 0.5 ml of the Standard Diluent Buffer into each tube (apart from the highest standard tube), including control well. Add 0.5 ml of the highest standard solution into the 1st tube and mix thoroughly. Transfer 0.5 ml from the 1st to 2nd tube, mix thoroughly, and so on.

Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once the standard has been reconstituted, it should be used within 15 mins. It is not recommended to reuse the reconstituted standard.



Wash Buffer: Dilute the concentrated Wash buffer 25-fold (1/25) with distilled water (i.e. add 30 ml of concentrated wash buffer into 720 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Avidin-HRP Working Solution Preparation:

1. Calculate the total volume of working solution required.
2. Dilute Avidin-HRP 100-fold with Avidin-HRP Diluent, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

C. Assay Protocol

Prepare all reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Set standard wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Immediately aliquot 50 µl of Avidin-HRP working solution to each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 30 mins.
4. Remove the cover and discard the solution. Wash the plate 3 times with Wash Buffer. *Fill each well completely with Wash buffer (300 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
6. Aliquot 90 µl of TMB Substrate into each well. Cover the plate with the plate sealer. Gently tap the plate to mix thoroughly. Incubate at 37°C for 15 minutes. The incubation time is for reference only, the optimal time should be determined by end user. Avoid exposure to light.
7. Aliquot 50 µl of Stop Solution into each well. It is important that the Stop Solution is mixed quickly and uniformly throughout the microplate to inactivate the enzyme completely.
8. Ensure that there are no fingerprints or water on the bottom of the plate, and that the fluid in the wells is free of bubbles. Measure the OD at 450 nm immediately.

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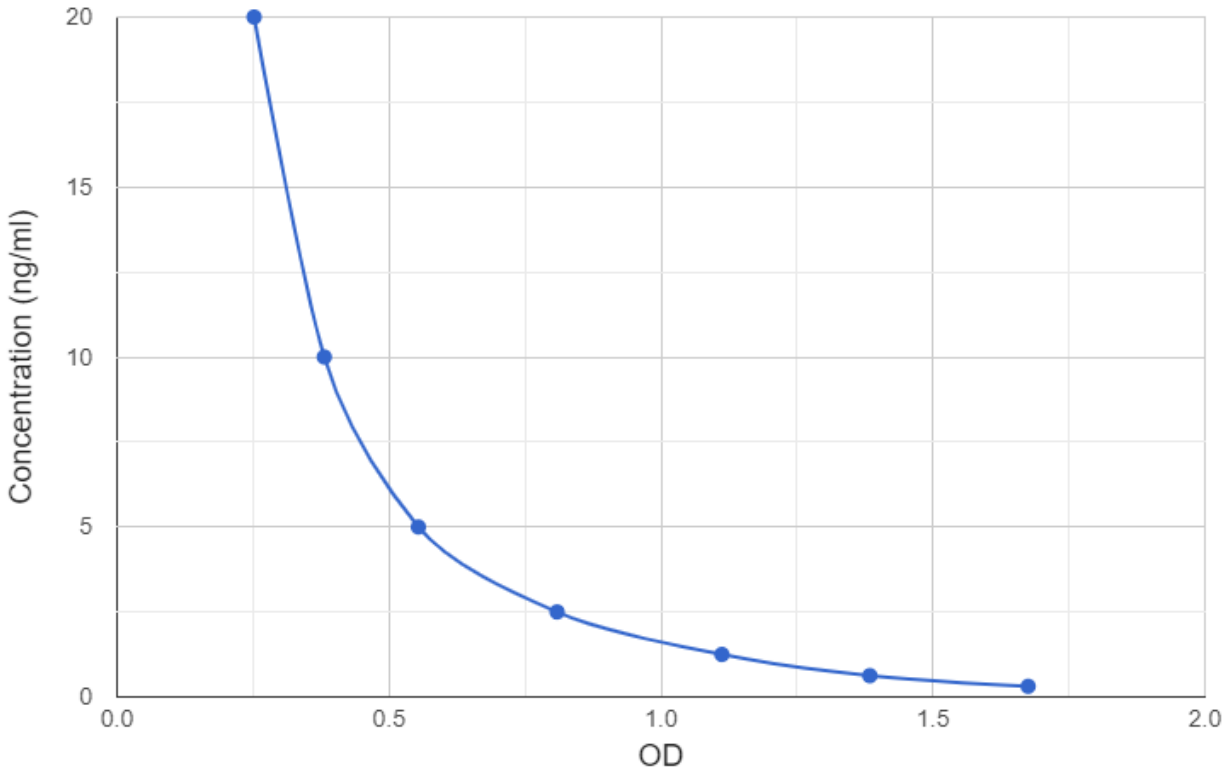
This assay is competitive, therefore there is an inverse correlation between the concentration of the sample and the OD measured. Create a graph with the log of the standard concentration (Y) and OD measured (X). Apply a best fit trendline through the standard points. Use this graph to calculate sample concentrations based on their OD values. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- The TMB substrate should be used under sterile conditions, and light exposure should be minimised. Unused substrate should be colorless, or a very light yellow in appearance. Do not discard any residual solution back into the vial.

D. Typical Data and Standard Curve

Typical Standard Curve Data is provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



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