Elabscience®

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Biotin quantitative Determination Kit

Catalog No: E-IR-R501

48T/96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Specification

- Sensitivity: 0.188 ng/mL (The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero).
- Detection Range: 0.313–20 ng/mL
- Specificity: This kit recognizes Biotin. No significant cross-reactivity or interference was observed.
- Repeatability: Coefficient of variation is <10%.

Test principle

This kit uses Competitive-method. The microplate provided in this kit has been pre-coated with Biotin. During the reaction, Biotin in the sample or standard competes with a fixed amount of Biotin on the solid phase supporter for sites on the Avidin-HRP specific to Biotin. Excess Avidin-HRP and unbound sample or standard are washed from the plate. Then a TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by adding Stop Solution and the color change can be measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of Biotin in samples can be calculated by comparing the OD of the samples with the standard curve.

Kit components & Storage

The unopened kit can be stored at 4℃ for 1 month. If the kit is not used within 1 month, store the items separately according to the following conditions since the kit is received.

Item	Specifications	Storage
Micro ELISA Plate (Dismountable)	8×12 or 8×6 *	-20℃, 12 months
Reference Standard	2/1 vial	
Concentrated Avidin-HRP	1 vial, 120 μL/60 μL *	-20℃(shading light), 12 months
Reference Standard & Sample Diluent	1 vial, 20 mL/12 mL *	4℃, 12 months
HRP Conjugate Diluent	1 vial, 14 mL	
Concentrated Wash Buffer (25×)	1 vial, 30 mL	
Substrate Reagent	1 vial, 10 mL	4℃(shading light)
Stop Solution	1 vial, 10 mL	4℃
Plate Sealer	5/3pieces *	
Product Description	1 copy	
Certificate of Analysis	1 copy	

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

The volume of reagents in partial shipments is a little more than the volume marked on the label, please use in measuring instead of directly pouring.

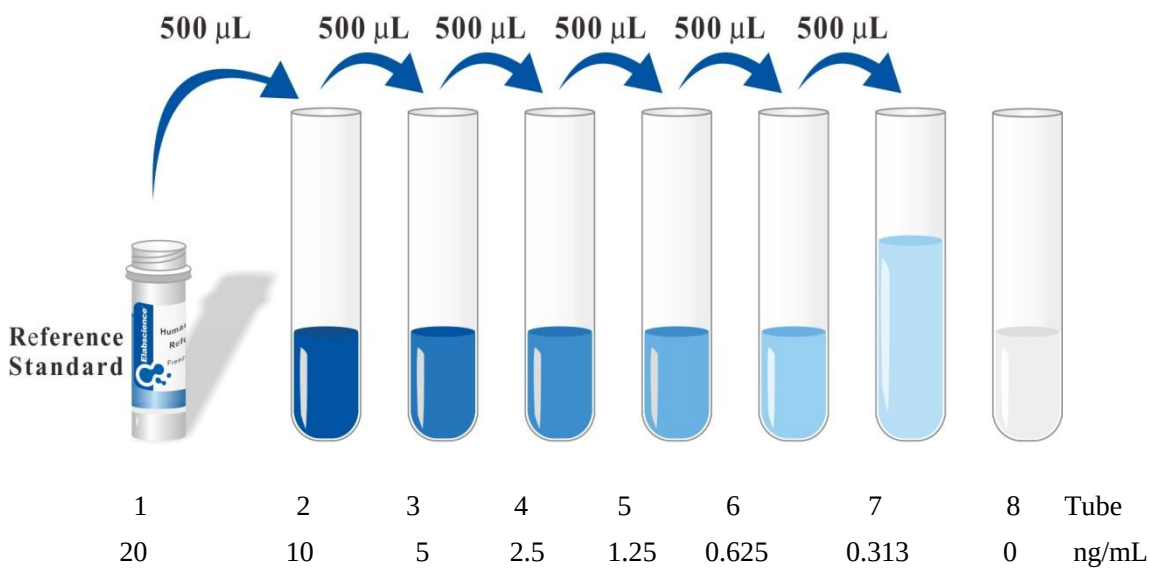
Other supplies required

Microplate reader with 450nm wavelength filter
High-precision transferpettor, EP tubes and disposable pipette tips
37°C Incubator, Deionized or distilled water
Absorbent paper

Reagent preparation

1. Bring all reagents to room temperature (18~25℃) before use. Preheat the Microplate reader for 15 min before OD measurement.
2. **Wash Buffer**: Dilute 30 mL of Concentrated Wash Buffer with deionized or distilled water to prepare 750 mL Wash Buffer. Note: if crystals have formed in the concentrate, warm it in 40℃ water bath and mix it gently until the crystals have completely dissolved.
3. **Standard working solution:** Centrifuge the standard at 10,000×g for 1 min. Add 1.0 mL of Reference Standard &Sample Diluent, let it stand for 10 min and turn it upside down for several times. After it dissolves fully, mix it thoroughly with a pipette. This reconstitution produces a stock solution of 20 ng/mL. Then make serial dilutions as needed. The recommended dilution gradient is as follows: 20, 10, 5, 2.5, 1.25, 0.625, 0.313, 0 ng/mL.

   Dilution method: Take 7 EP tubes, add 500 uL of Reference Standard & Sample Diluent to each tube. Pipette 500 uL of the 20 ng/mL stock solution to the first tube and mix up to produce a 10 ng/mL stock solution. Pipette 500 uL of the solution from former tube to the latter one in order according to this step. The illustration below is for reference. Note: the last tube is regarded as blank. Don't pipette solution to it from the former tube.



4. **Concentrated Avidin-HRP:** Calculate the required amount before experiment (50 μL/well). In actual preparation, more account of 100~200 μL should be prepared. Dilute the Concentrated Avidin-HRP to the working concentration with HRP Conjugate Diluent (1:99).

Washing Procedure

1. **Automated washer:** add 350 μL wash buffer into each well, the interval between injection and suction should be set about 60s.
2. **Manual wash:** add 350 μL wash buffer into each well, soak it for 1~2 min, suck(no inside wall touching) or get rid of liquid within the micro ELISA plate and pat it dry on thick clean absorbent paper.

Assay procedure (A brief assay procedure is on the 6th page)

Bring all reagents to room temperature (18~25℃) before use. **All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.**

1. Add **Standard working solution** of different concentrations to the first two columns: Each concentration of the solution is added into two wells side by side (50 uL for each well). Immediately add 50μL of **Avidin-HRP working solution** to each well. Cover the plate with sealer provided in the kit. Incubate for 30 min at 37℃. Note: solutions should be added to the bottom of micro ELISA plate well, avoid touching the inside wall and foaming as possible.
2. Aspirate or decant the solution from each well， add 350 uL of **wash buffer** to each well. Soak for 1~2 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times. Note: a microplate washer can be used in this step and other wash steps.
3. Add 90 μL of **Substrate Reagent** to each well. Cover with a new plate sealer. Incubate for about 15 min at 37°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30min.
4. Add 50 μL of **Stop Solution** to each well. Note: the order to add stop solution should be the same as the substrate solution.
5. Determine the optical density (OD value) of each well at once, using a micro-plate reader set to 450 nm.
6. After experiment, put all the unused reagents back into the refrigerator according to the specified storage temperature respectively until their expiry.

Important Notes

1. **Storage**: All the reagents in the kit should be stored following the instructions. Exposure of reagents to strong light should be avoided in the process of incubation and storage. All the taps of reagents should be tightened to prevent evaporation and microbial contamination, or erroneous results may occur.
2. **ELISA Plate**: Little water-like substance may appear in the ELISA Plate just opened, this is normal and will not have any impact on the experiment results. Keep the remaining plates in spare aluminum foil bag, and keep it at temperature suggested before.
3. **Add Sample**: The interval of sample adding between the first well and the last well should not be too long, otherwise will cause different pre-incubation time, which will significantly affect the experiment's accuracy and repeatability. The interval should be controlled within 10 min. Parallel measurement is recommended.
4. **Incubation**: To prevent evaporation, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to keep uncovered for extended periods between incubation steps. Do not let the strips dry at any time during the assay. Strict compliance with the given incubation time and temperature.
5. **Washing**: The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be pat dry against absorbent paper in the washing process. But don't put absorbent paper into reaction wells directly. Note that clear the residual liquid and fingerprint in the bottom before measurement, so as not to affect the microtiter plate reader.
6. **Reagent Preparation**: As the volume of Concentrated Avidin-HRP is very small, liquid may adhere to the tube wall or tube cap when being transported. It is recommended to hand-throw it or centrifuge it for 1 min at 1000 rpm. Please pipette the solution for 4-5 times before pippeting. Please carefully reconstitute Standards, Avidin-HRP working solution according to the instructions. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 μL for each pipetting. Do not reuse standard solution, Avidin-HRP working solution, which have been diluted. If you need to use standard repeatedly, you can divide the standard into small pack according to the amount of each assay, keep them at -20~ -80°C and avoid repeated freeze/thaw cycles.
7. **Reaction Time Control:** Please control reaction time strictly following this manual!
8. **Substrate:** Substrate Solution is easily contaminated. Protect it from light.
9. **Mixing:** You'd better use microoscillator at the lowest frequency, as sufficient and gentle mixing is particularly important to reaction result. If there is no microsocillator available, you can knock the ELISA plate frame gently with your finger before reaction.
10. **Security:** Please wear lab coats and latex gloves for protection. Especially detecting samples of blood or other body fluid, please perform following the national security columns of biological laboratories.
11. Do not use component from different batches of kit (washing buffer and stop solution can be an exception).

12. Change pipette tips between adding of each standard level, sample, and reagent to avoid cross-contamination. Use separate reservoirs for each reagent. **Otherwise, the results will be inaccurate!**

Calculation of results

Average the duplicate readings for each standard and samples. Plot a four-parameter logistic curve on log-log graph paper, with standard concentration on the x-axis and OD values on the y-axis.

If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor. If the OD of the sample surpasses the lower limit of the standard curve, you should re-test it after appropriate dilution. The actual concentration is the calculated concentration multiplied dilution factor.

SUMMARY

1. Add 50 μL standard or sample to each well.
2. Immediately add 50 μL Avidin-HRP to each well.
3. Incubate for 30 min at 37℃
4. Aspirate and wash 5 times
5. Add 90 μL Substrate Reagent. Incubate 15 min at 37℃
6. Add 50 μL Stop Solution. Read at 450 nm immediately.
7. Calculation of results