（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-K1105-M

Specification：48T(32 samples)/96T(80 samples)

Measuring instrument：Microplate reader (500 nm)

Detection range：12.60-300.00 μmol/L

Elabscience® Biotin Colorimetric Assay Kit (HABA Method)

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

If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623

Tell: 1-832-243-6086

Fax: 1-832-243-6017

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.

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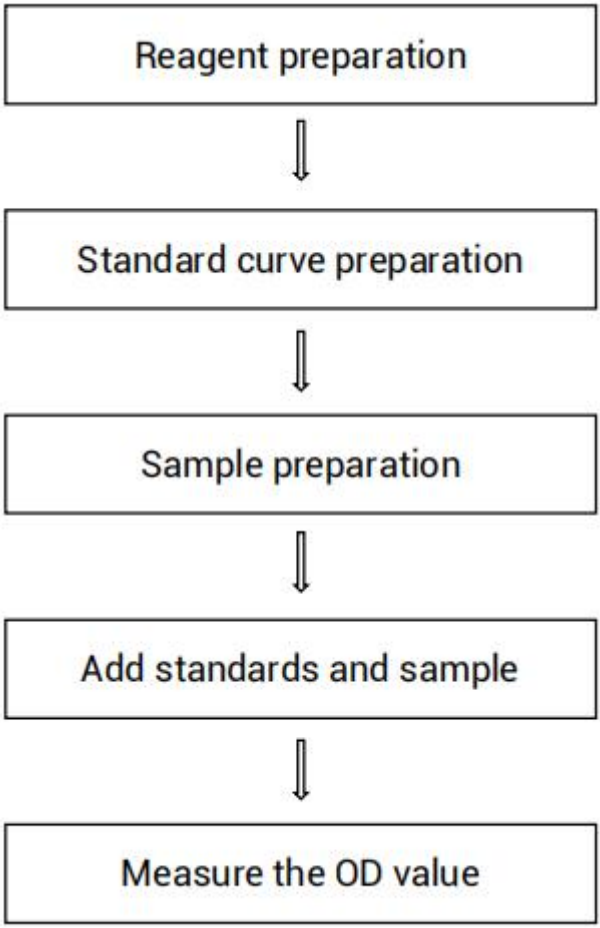
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Assay summary



Intended use

This kit can be used to measure the biotin content of antibodies or proteins that have been biotinylated.

Detection principle

Biotin is an important biological molecule that is widely used to label antibodies or other proteins in biotinylated reactions.

The detection principle: HABA binds to streptavidin, forming a complex that exhibits a characteristic absorbance maximum at 500 nm. Owing to the substantially higher affinity of biotin (Kd = 1 × 10^-15 M) for streptavidin relative to that of HABA (Kd = 5.8×10^-6 M), the presence of biotin in the sample competitively displaces HABA from the streptavidin binding sites. This displacement results in a concentration-dependent decrease in absorbance at 500 nm.

Consequently, the absorbance decrement is inversely proportional to the biotin concentration in the test sample, enabling accurate and rapid quantification of free biotin or the extent of protein/antibody biotinylation.

Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL × 1 vial	30 mL 1vial	-20℃, 12 months, shading light
Reagent 2	Substrate A	0.02 mL × 1 vial	0.04 mL × 1 vial	-20℃, 12 months, shading light
Reagent 3	Substrate B	0.6 mL × 1 vial	1.2 mL × 1 vial	-20℃, 12 months, shading light
Reagent 4	Alkali Reagent	2 mL × 1 vial	4 mL × 1 vial	-20℃, 12 months, shading light
Reagent 5	30 mmol/L Standard Solution	0.02 mL × 1 vial	0.02 mL × 1 vial	-20℃, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement
	Plate Sealer	2 pieces		
	Sample Layout Sheet	1 piece		

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:
Microplate reader (500 nm), Incubator

Reagent preparation

① Equilibrate all reagents to 25°C before use.

② The preparation of substrate A dilution solution：

Before testing, please prepare sufficient substrate A dilution solution

according to the test wells. For example, prepare 100 μL of substrate A dilution solution (mix well 10 μL of alkali reagent and 90 μL of buffer solution). Aliquoted storage at -20°C for 7 days protected from light.

③ The preparation of substrate A working solution：
Before testing, please prepare sufficient substrate A working solution according to the test wells. For example, prepare 1000 μL of substrate A working solution (mix well 100 μL of substrate A and 900 μL of substrate A dilution solution, yellow and transparent is effective). Store at -20°C for 1 day protected from light.

④ The preparation of substrate B working solution：
Before testing, please prepare sufficient substrate B working solution according to the test wells. For example, prepare 200 μL of substrate B working solution (mix well 100 μL of substrate B and 100 μL of double distilled water). Aliquoted storage at -20°C for 7 days protected from light.

⑤ The preparation of reaction working solution：
For each well, prepare 40 μL of reaction working solution (mix well 20 μL of substrate A working and 20 μL of substrate B working, orange and transparent is effective). Aliquoted storage at -20°C for 1 day protected from light.

⑥ The preparation of 300 μmol/L standard solution：
Before testing, please prepare sufficient 300 μmol/L standard solution. For example, prepare 1000 μL of 300 μmol/L standard solution (mix well 10 μL of 30 mmol/L standard solution and 990 μL of buffer solution). Aliquoted storage at -20°C for 7 days protected from light.

⑦ The preparation of standard curve：
Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 300 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 60, 90, 120, 150, 180, 210, 300 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**60**	**90**	**120**	**150**	**180**	**210**	**300**
300 μmol/L Standard (μL)	0	40	60	80	100	120	140	200
Buffer Solution (μL)	200	160	140	120	100	80	60	0

Sample preparation

Antibodies or proteins labeled with biotin: detect directly.

Operating steps

① Standard well: add 20 μL of standard with different concentrations into the standard wells.

Sample well: add 20 μL of sample into the sample wells.

② Add 40 μL of reaction working solution into each well.

③ Add 140 μL of buffer solution into each well.

④ Measure the OD values of each well at 500 nm with microplate reader.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean OD value of the blank (Standard #①) from all standard readings. This is the absoluted OD value.

3. Plot the standard curve by using absoluted OD value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

$$\text{Biotin content (μmol/L)} = (\Delta A - b) \div a \times f$$

[Note]

ΔA: $OD_{blank} - OD_{sample}$

f: Dilution factor of sample before test.

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three antibodies samples were assayed in replicates of 20 to determine precision within an assay (CV = Coefficient of Variation).

Parameters	Standard 1	Standard 2	Standard 3
Mean (μmol/L)	50.00	100.00	150.00
%CV	1.2	1.4	3.4

Inter-assay Precision

Three antibodies samples were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Standard 1	Standard 2	Standard 3
Mean (μmol/L)	50.00	100.00	150.00
%CV	3.5	4.5	6.0

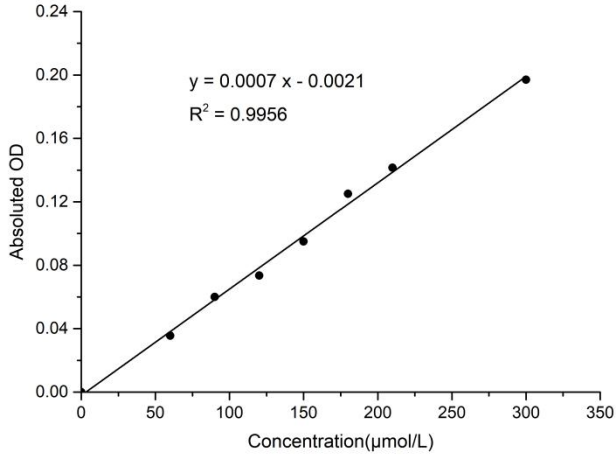
Sensitivity

The analytical sensitivity of the assay is 12.60 μmol/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the OD value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	60	90	120	150	180	210	300
OD	0.272	0.240	0.208	0.195	0.179	0.145	0.132	0.072
	0.274	0.234	0.217	0.203	0.176	0.150	0.130	0.079
Average OD	0.273	0.237	0.213	0.199	0.178	0.148	0.131	0.076
Absoluted OD	0.000	0.036	0.060	0.074	0.095	0.125	0.142	0.197



Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.