TECHNICAL MANUAL

Biotin Colorimetric Assay Kit

- **SKU CODE:** MAES0511
- **SIZE:** 48 Tests
- **DETECTION PRINCIPLE:** Assay Kit
- **RUO:** Research-Use-Only

Revised: 01/2026

1. Assay summary

- Reagent preparation
- Standard curve preparation
- Sample preparation
- Add standards and samples
- Measure optical density at 500 nm

2. Intended use

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

3. Detection principle

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

The detection principle: Biotin can compete with avidin for binding to the dye, causing a decrease in the absorbance value at 500 nm. The biotin content is calculated based on this decrease in absorbance.

4. Kit components & storage

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
Reagent 1	Buffer Solution	15 mL x 1 vial	30 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 2	Substrate A	0.02 mL x 1 vial	0.04 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 3	Substrate B	0.6 mL x 1 vial	1.2 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 4	Alkali Reagent	2 mL x 1 vial	4 mL x 1 vial	-20 °C, 12 months, shading light
Reagent 5	30 mmol/L Standard Solution	0.02 mL x 1 vial	0.02 mL x 1 vial	-20 °C, 12 months, shading light
	Microplate	48 wells	96 wells	No requirement

Item	Component	Size 1(48 T)	Size 2(96 T)	Storage
	Plate Sealer	2 pieces	2 pieces	

5. Materials prepared by users

Instruments:

Microplate reader (500 nm), Incubator

6. Reagent preparation

1. Allow all reagents to equilibrate to 25 °C before use.
2. **Preparation of substrate A dilution solution:** Before testing, 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). Store aliquots at -20 °C for 7 days protected from light.
3. **Preparation of substrate A working solution:** Before testing, 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, should be yellow and transparent). Store at -20 °C for 1 day protected from light.
4. **Preparation of substrate B working solution:** Before testing, 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). Store aliquots at -20 °C for 7 days protected from light.
5. **Preparation of reaction working solution:** For each well, prepare 40 µL of reaction working solution (mix well 20 µL of substrate A working solution and 20 µL of substrate B working solution, should be orange and transparent). Store aliquots at -20 °C for 1 day protected from light.
6. **Preparation of 300 µmol/L standard solution:** Before testing, 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). Store aliquots at -20 °C for 7 days protected from light.
7. **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.

7. Sample preparation

Antibodies or proteins labeled with biotin: detect directly.

8. 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.

9. 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 absolute OD value.

3. Plot the standard curve using absolute 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:

Biotin content (μmol/L) = (ΔA - b) ÷ a × f

[Note]

ΔA: OD_{blank} - OD_{sample}

f: Dilution factor of sample before test.

10. Appendix I Performance Characteristics

Parameter:

Intra-assay Precision

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

Intra-assay Precision

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.

Inter-assay Precision

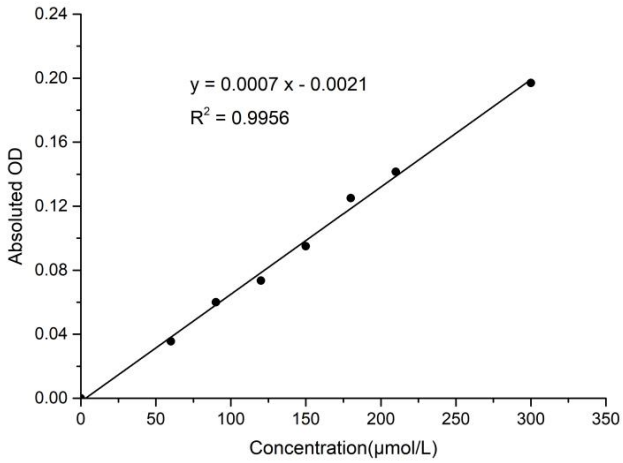
Parameters	Standard 1	Standard 2	Standard 3
Mean (µmol/L)	50.00	100.00	150.00
%CV	3.5	4.5	6.0

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.

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), the standard curve and data are provided below for reference only:



Standard curve

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

11. Statement

1. This assay kit is for Research Use Only. Assay Genie will not be responsible for any arising problems or legal responsibilities caused by using the kit for clinical diagnosis or other purposes.
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 performed on the sample.

5. It is recommended to perform a pre-test if your sample is not listed in the instruction manual.

6. The experimental results are closely related to the condition of reagents, operations, environment and other factors. Assay Genie will guarantee the quality of the kits only, and will NOT be responsible for the sample consumption caused by using the assay kits. It is advisable to calculate the possible usage of sample and reserve sufficient samples before use.

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

Manufacturers Statement: This final kit system is assembled and quality-released by Assay Genie Limited.