

QuantTag™ Biotin Quantitation Kit

Cat. No.	BDK-2000
Storage	Store kit at room temperature.
Description	The QuantTag Biotin Quantitation Kit is designed to determine the amount of free biotin in solution or the number of biotins attached to proteins, nucleic acids, or other macromolecules. The kit reagents chemically react with free or bound biotin, producing a colored product that can be quantified using a spectrophotometer. The absorbance is measured in the visible spectrum, allowing the use of plastic cuvettes or microtitre plates.

Kit Components[†]

Product	Volume
QuantTag Reagent 1	87.5 ml
QuantTag Reagent 2	87.5 ml
QuantTag Reagent 3	87.5 ml
QuantTag Biotin Standard	1 ml: 0.5 mM

The kit contains sufficient reagents to perform 25 assays using the 1 ml protocol (including a standard curve for each) or 250 assays using the 0.1 ml protocol.

[†] Avoid contact with skin and eyes. If contact occurs, flush immediately with water.

Principle

The assay can be done using either of the two protocols. The choice of protocol will depend on the size of the cuvette used in the assay. The sample to be tested (containing an unknown amount of biotin) is reacted with the kit reagents along with standards containing known amounts of biotin. The absorbance readings of the known samples are plotted producing a standard curve. The absorbance of the test sample is located on the standard curve indicating the amount of biotin present.

Protocol

The assay can be done using either of the two protocols. The choice of protocol will depend on the size of the cuvette used in the assay.

1 ml Assay:

The protocol for the 1 ml assay is intended for use with 1 ml cuvettes. Reactions may be performed directly in the cuvette or may be performed in a test tube and transferred to a cuvette prior to measuring the absorbance.

1. Prepare the QuantTag working solution as follows: Combine 0.5 ml of Reagent 1, 0.5 ml of Reagent 2, and 50 μ l of Reagent 3 for each sample. (The yellow Reagent 3 turns pale orange upon mixing with Reagents 1 and 2.) The total amount needed is 6 ml (for the standards) plus 1 ml for each of the test samples. The working solution can be used for up to eight hours.

2. Prepare the biotin standards as follows: Add 1 μ l, 2 μ l, 5 μ l, 10 μ l, and 20 μ l of the Biotin Standard Solution to each of five cuvettes. These standards will contain 0.5 nmol, 1 nmol, 2.5 nmols, 5 nmols, and 10 nmols of biotin, respectively. To a sixth cuvette, add no biotin (this will be used to "zero" the spectrophotometer).

3. Adjust the concentration of the biotinylated molecule so that the number of biotins likely falls within the range of the standard curve. Two different concentrations or volumes of the test sample may be run simultaneously. (See Note A)

4. Add 20 μ l or less of the test sample to a cuvette. Most common buffers do not interfere with the assay (See Note B). However, accuracy may be improved by including a control sample containing unbiotinylated test molecule (e.g. DNA or protein) in a similar solution as the biotinylated sample. The absorbance reading of this control can then be subtracted from the absorbance reading of the test sample.

5. To each cuvette, add 1 ml of the QuantTag working solution prepared in Step 1 and incubate at room temperature for 30 minutes. The colored reaction product is stable for several hours.

6. Measure the absorbance of the test sample and biotin standards at 535 nm. Use the standard containing no biotin, to "zero" the instrument.

7. To determine the nmols of biotin in test samples, use biotin standard values to plot the standard curve with nmols of biotin on the X-axis and absorbance on the Y-axis, as shown in Note C, Fig. 1. To determine the number of biotins per biotinylated molecule see Note C.

0.1 ml Assay:

This protocol is for use with 0.1 ml cuvettes or microtitre plates. When using the 0.1 ml protocol, consideration must be given to sample volume. See Note D.

1. Prepare the QuantTag working solution as follows: Combine 50 μ l of Reagent 1, 50 μ l of Reagent 2, and 5 μ l of Reagent 3 for each sample. (The yellow Reagent 3 turns pale orange upon mixing with Reagents 1 and 2.) The total amount needed is 600 μ l (for the standards) plus 100 μ l for each of the test samples. The working solution can be used for up to eight hours.

2. For the 0.1 ml assay, prepare the biotin standards as follows: Dilute 1 μ l of the Biotin Standard Solution into 9 μ l of water to generate a 1:10 dilution. Pipet the amounts of 1:10 dilution and original undiluted Biotin Standard Solution into 6 cuvettes as shown in the table below. Adjust the volume of each biotin standard to 5 μ l with water. The amount of biotin in each standard, in nmols, is indicated in the table.

See reverse side for additional information.

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Biotin Standard	1:10 dilution of Biotin Solution (μl)	Undiluted Biotin Solution (μl)	Water (μl)	Total Volume (μl)	nmols of Biotin
1			5	5	0
2	1		4	5	0.05
3	2		3	5	0.1
4	5			5	0.25
5		1	4	5	0.5
6		2	3	5	1

3. Adjust the concentration of the biotinylated molecule being tested so that the estimated amount of biotin likely falls within the range of the standard curve. Two different concentrations of the test sample may be run simultaneously. (See Note A)

4. Add 5 μl of the test sample(s) to a cuvette. Most common buffers do not interfere with the assay (See Note B). However, accuracy may be improved by including a control sample containing unbiotinylated test molecule (e.g. DNA or protein) in a similar solution as the biotinylated sample. The absorbance reading of this control can then be subtracted from the absorbance reading of the test sample.

5. To each cuvette, add 0.1 ml of the QuantTag working solution prepared in Step 1 and incubate at room temperature for 30 minutes. The colored reaction product is stable for several hours.

6. Measure the absorbance of the test sample(s) and biotin standards at 535 nm. Use the standard containing no biotin to “zero” the instrument.

7. To determine the nmols of biotin in the test samples, use biotin standard values to plot the standard curve with nmols of biotin on the X-axis and absorbance on the Y-axis, as shown in Note C, Fig. 1. To determine the number of biotins per biotinylated molecule, see Note C.

Notes:

Note A: For most biotinylated proteins, assume 2 to 30 nmols of biotin per nmol of protein. In general, 5–20 μl of a 1 mg/ml solution of biotinylated protein will likely fall in the range of the standard curve when using the 1 ml assay. For the 0.1 ml assay, 0.5–5 μl of a 1 mg/ml solution is generally adequate.

Some biotinylated proteins at high concentration may be insoluble in the QuantTag working solution. If the biotinylated protein is not dissolved after the 30 minute incubation with QuantTag solution, repeat the assay with a lower protein concentration.

For most biotinylated DNA, assume one biotin per 10–30 base pairs or 33–100 biotins per kilobase pairs. In general, 10–20 μl of a 1 μg/μl solution of biotinylated DNA will likely fall within the range of the standard curve when using the 1 ml assay. For the 0.1 ml assay, 1–5 μl of a 1 μg/μl solution is usually enough.

Note B: The following buffers and compounds were found not to interfere with the QuantTag assay (tested by adding 20 μl of 100 mM solution to 1 ml QuantTag working solution):

Azide	EDTA	Phosphate
Bis Tris	EPDS	PIPES
Bis Tris Propane	HEPES	Tricine
Borate	Imidazole	Tris
carbonate	MES	
Citrate	MOPS	

Note C: To determine the number of biotins per biotinylated molecule, first determine the amount of biotinylated molecule tested, in nmols, by using the following equation:

$$\frac{A \times B \times 1000}{C} = \text{nmols of biotinylated molecule}$$

where:

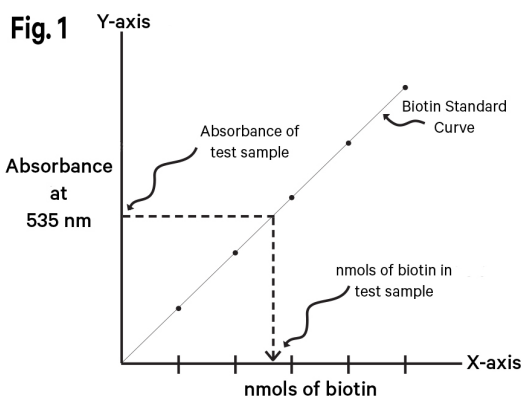
A = concentration of test molecule added (μg/μl)

B = volume of test molecule added (μl)

C = molecular weight of test molecule (μg/μmol)

Knowing the nmols of biotin in the test sample (n) (derived from standard curve) and the nmols of test molecule in the assay (m), the number of biotins on the molecule can be calculated:

$$\frac{n}{m} = \text{biotins/molecule}$$



Note D: When using the 1 ml assay, the variation in volumes from 1 μl to 20 μl of the standard biotin solutions and test samples introduce a variation in the total reaction volumes of ≥ 2%. This does not significantly affect the absorbance readings. When using the 0.1 ml assay, however, this difference in volume may become significant. To reduce this variation and to avoid further dilution of the QuantTag solution, use the smaller volumes of biotin standards and test solutions shown in the table under the protocol for the 0.1 ml assay.