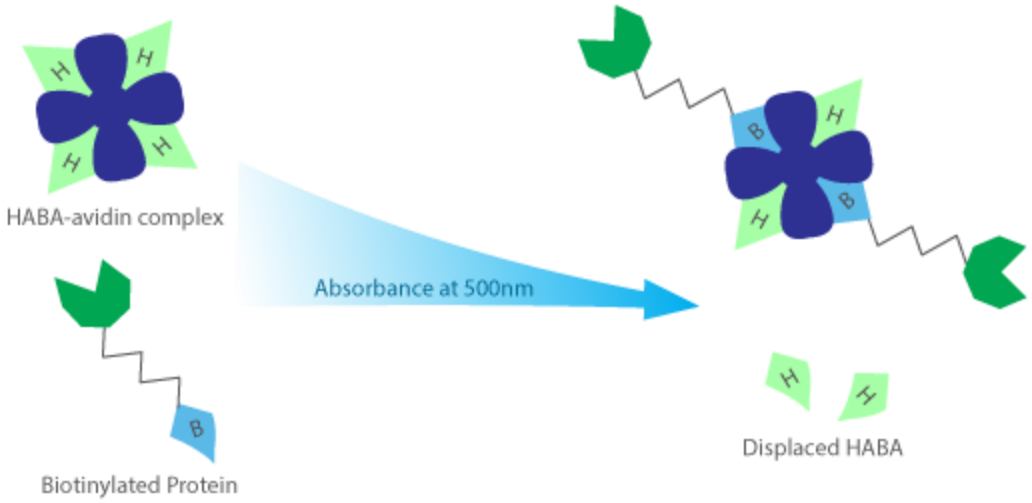
Biotin Quantitation Kit

Component	Product size	storage
	BP-50060	
HABA-avidin premix	18 vials	-20°C

Overview

The BroadPharm Biotin Quantitation kit enables a quick estimation of the mole-to-mole ratio of biotin to protein. To quantify biotin label incorporation, a solution containing the biotinylated protein is added to a mixture of HABA and avidin. Because of its higher affinity for avidin, biotin displaces HABA from its interaction with avidin and the absorption at 500 nm decreases proportionately. By this method, an unknown amount of biotin present in a solution can be evaluated in a single cuvette by measuring the absorbance of the HABA-avidin solution before and after addition of the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample.

Refer to BroadPharm kits Sulfo-NHS-LC-Biotin kit (BP-50058), Sulfo-NHS-Biotin (BP-50056), and NHS-PEG4-Biotin (BP-50054) for your biotinylating needs.



Important Product Information

- The biotin-labeled protein must be desalted or dialyzed to remove all traces of nonreactive and hydrolyzed biotinylation agents before performing the quantitation assay.
- Samples must be in one of the recommended buffers (PBS or TBS, see Reagent Preparation Section) for the assay. Avoid buffers containing potassium (such as Modified Dulbecco's PBS), which will cause precipitation in the assay. Buffers that may interfere with labeling should not be used unless first validated by comparing to results using PBS or TBS.
- Slight color variation between the HABA-avidin premix microtubes does not affect product performance.

A. Reagent Preparation

- Allow all components to reach room temperature.
- Add 1 ml of DI water to reconstitute a vial of HABA-avidin premix (component A), mix, allow for 5 minute wait time, and mix again. Pulse spin centrifuge to bring liquid to the bottom of the vial.
- Suggested protein sample size ~ 100 kD, concentration 1 mg/ml with 5x biotin to protein ratio.

Note: unused HABA-avidin solution is stable at 4^0C for 2 weeks.

B. Procedures for Quantitation:

Cuvette Format for quantitation of moles of biotin per mole of protein

1. In a 1 ml cuvette add 900 µl of HABA-avidin solution reconstituted above. Spec. at 500 nm, record as A_0.
2. Add 100 µl of biotinylated protein sample to the cuvette containing HABA-avidin, from step above, and mix well (ensure no bubbles collect on cuvette wall). Allow for a 2-minute wait time, spec. the sample at 500 nm and record the value as A_1. (If the reading is ≤ 0.3, dilute the sample and repeat the assay. Dilutions must be accounted in the calculation step.)

Microplate Format quantitation of moles of biotin per mole of protein

1. In a microplate well, add 180 µl of reconstituted HABA-avidin premix + 20 µl of 1x PBS or water, and pipet gently up and down to mix with a micropipette, or use an orbital shaker 400 rpm, 1 minute. This well's reading will be recorded as A_1 (reading instruction step 4).
2. To another well, add 180 µl of reconstituted HABA-avidin premix + 20 µl of biotinylated protein sample, and pipet gently up and down to it with a micropipette, or use an orbital shaker 400 rpm, 1 minute. The reading for this well will be recorded as A_1 (reading instruction step 4).
3. Allow a two-minute waiting period.
4. Read the two wells at the same time, absorbance at 500 nm.

Note:

C. Calculations for Moles of Biotin Per Mole of Protein

Note: Visit our website Broadpharm.com for online B/P ratio calculator.

$$\text{Biotin/protein} = \frac{mmol\ per\ mL\ biotin\ in\ reaction\ mixture}{mmol\ per\ mL\ protein\ in\ original\ sample}$$

$$= \frac{(0.9\times A_0 - A_1)\times DF\times 10\times MW}{34000\times b\times C}$$ (cuvette assay, b=1)

or

$$= \frac{(A_0 - A_1)\times DF\times 10\times MW}{34000\times b\times C}$$ (microplate assay, b=0.5)

- A_0: HABA/avidin reaction mixture absorbance at 500 nm
- A_1: HABA/avidin/biotin reaction mixture absorbance at 500 nm
- 34000 is the HABA/avidin samples absorptivity or extinction coefficient at 500 nm.
- b is the cell path length expressed in centimeters (cm). A 10mm square cuvette has a path length of 1.0 cm. In the microplate format with the recommended volumes, the path length is typically 0.5 cm.
- C is the sample concentration (mg/ml).

- MW Molecular weight (MW) of the protein (e.g., HRP = 40,000; IgG =150,000)
- DF: Dilution factor, if the sample is diluted before adding to the HABA/avidin reaction mixture. DF = 1, if biotinylated protein is not diluted outside of the given procedure above.