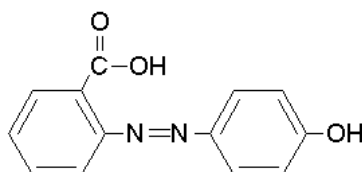


HABA, Biotin detection agent

Product Description

Orange dye allowing the colorimetric quantity of biotin.

Catalog number:	UP05361D, 2g
Name:	HABA
Formula:	2-(4'-HydroxyAzoBenzene)Benzoic Acid
CAS[1634-82-8],	MW 242.24
Storage:	Store at Room Temperature



Scientific and Technical Information

- HABA reagent offers an easy way to determine the biotin content of a solution over a wide range of pH and salt concentration. An interesting application is the estimation of the biotin content of protein after a biotinylation of biomolecules.
- The HABA (2-Hydroxyazobenzen-4'-Carboxylic Acid) when binding to avidin, produces a yellow-orange colored complex which absorbs at 500 nm. Biotin, a vitamin that has a very high affinity to avidin ($K_a=10^{-15}M^{-1}$), displace easily the HABA from the complex, causing the absorbance to decrease. The biotin present in a sample can thus be determined from interpolation from a standard curve of free biotin, then the number of moles of biotin per mole of biotinylated protein can be calculated.
- HABA method can be applied to both purified proteins or complex mixtures.

Directions for Use

Protocol of Biotin dosage

This is a standard protocol for the dosage of biotin in solution (1)

Preparation of the reagents solution:

- 1- HABA Reagent: 24.2 mg HABA in 9.9 ml H₂O, then 100 μ l 1N NaOH. The HABA solution can be stored at +4°C or frozen in aliquotes. In case of a insufficient dissolution of HABA, you can add 100 μ l 1N NaOH and eventually filtrated to remove particules.
- 2- Avidin-HABA Reagent: 10 mg of avidin (#UP39860D) + 600 μ l of HABA Reagent; complete to 20ml of PBS. Use immediately, or eventually store this solution at +4°C for 1 week use.

FT-UP05361

Tube protocol:

- 1- Pipette 900 µl of the Avidin-HABA Reagent in a 1 ml cuvette.
- 2- Measure the OD at 500 nm. The OD_{500nm} should be 0.9-1.3.
- 3- Add 100µl of biotinylated sample and mix. Measure the OD at 500 nm . The OD_{500nm} should be stable.
If the OD is below 0.3, the sample should be diluted (due to an excess of biotin who gives a non significative absorbance at 500nm).

Treatment with pronase:

If the sample contains a highly biotinylated biomolecules, it should be treated with pronase to improve the availability for avidin.

- 1- Prepare a pronase solution at 1% in distilled water.
- 2- Heat 100 µl of biotinylated sample at 56°C for 10 min.
- 3- Add 10 µl of 1% pronase solution on sample and incubate overnight at room temperature.
- 4- Realize the test here before.

Determination of the number of biotin per protein:

Three data are necessary for the calculation:

- OD_{500nm} of the Avidin-HABA Reagent = **DO1**
- OD_{500nm} of the Biotin sample reaction mixture = **DO2**
- Molar concentration of the biotinylated sample = **P** (mM)

Calcul

Net OD _{500nm}	DA⁽¹⁾ = (0.9x DO1) – (DO2)	0.9 = dilution factor of Avidin -HABA with sample
µmol biotin per ml reaction mixture	C = DA / 34	34 = mM extinction coefficient at 500 nm
mmol biotin / ml reaction mixture	B = C x10xd	d = dilution factor of biotinylated sample
Ratio Biot / Prot	B/P	

(1) In the case of a colored biotinylated sample, her absorbance (**DO3**) should be measured and correction done as follow:

$$DA = (0.9x DO1)+DO3 - (DO2)$$

Other Information

Literature

- 1- Green N.M. Avidin. In Adv. in Protein Chemistry. Academic Press, New York. 1975, 29, 85-133
- 2- Savage, M.D., Mattson, G., Desai, S., Nielander, G.W., Morgensen, S., and Conklin, E.J. (1992). Avidin-Biotin Chemistry: A Handbook. Rockford, IL: Pierce Chemical Co. (Product #15055)
- 3- Janolino, V.G. et al. (1996). A spectrophotometric assay for biotin -binding sites of immobilized avidin. App. Biochem. and Biotech. 56, 1-7.
- 4- Green, N.M., Methods in Enzymology, Vol. 18A, p418 (1970).

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