FT-05361D


# HABA, Biotin detection agent

*Orange dye allowing the colorimetric quantity of biotin.*

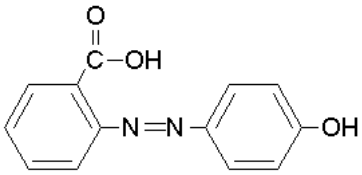
## Description

**Catalog number:** UP05361E, 10 g

**Name:** **HABA**

2-(4'-HydroxyAzoBenzene)Benzoic Acid
CAS [1634-82-8]; **MW 242.24**

**Storage:** Store at Room Temperature (Z)




## 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 (Ka=$10^{15}M^{-1}$), 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 µl 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 µl 1N NaOH and eventually filtrated to remove particules.

2- Avidin-HABA Reagent : 10 mg of avidin (#UP39860D) + 600µl of HABA Reagent; complete to 20ml of PBS. Use immediately, or eventually store this solution at +4°C for 1 week use.

**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 that 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 avaibility 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 $OD_{500nm}$ | **ΔA**(1) = | (0.9x **DO1**) – (**DO2**) | 0.9 = dilution factor of Avidin-HABA with sample |
| µmol biotin per ml reaction mixture | **C=** | **ΔA** / 34 | 34500 M-1 = extinction coefficient at 500 nm |
| mmol biotin / ml sample | **B=** | **C**x10x d | d = dilution factor of biotinylated sample |
| Molar Ratio of Biotin / Protein | | B/P | |

(1) In the case of a colored biotinylated sample, it's absorbance (**DO3**) should be measured and correction performed as follows:
**ΔA =** (0.9x **DO1**)+**DO3** – (**DO2**)

**Physical data - Extinction coefficients ($M^{-1}$ $cm^{-1}$) .**

| Molecule | Abs.max (nm) | 282nm | 350nm | 500nm |
|---|---|---|---|---|
| Avidin | 282 | 25000 | 0 | 0 |
| HABA | 350 | 2800 | 20500 | 600 |
| Avidin-HABA | 500 | - | 2000 | 34500 |

**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 #373790)
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).

# Other information

**Related products**

Streptavidin-AP #UP39588 Chromalink-Biotin (PEO spacer, color tag) #CE9601)



For any question, please ask Uptima or your local distributor.

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