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ProductInformation

HABA/AVIDIN REAGENT

Product No. **H 2153**

Store at 2-8 °C

Product Description

HABA/Avidin Reagent is suitable for the spectrophotometric determination of biotin levels in a diverse range of sample types. This is a modified version of a literature preparation which obviates the need for additional supplements to avoid non-specific or ionic interactions with most proteins.∗

∗Except in special cases such as lectins that require supplementation with as appropriate sugar in the range of 0.1 M to 0.5 M.

When reconstituted with 10 ml deionized water, each vial will yield the following solution, pH approx. 7.3:

- 0.3 mM HABA (4-Hydroxyazobenzene-2-carboxylic acid)
- 0.45 mg/ml avidin
- 0.3 M NaCl
- 0.01 M HEPES (N-[2-Hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid], a buffer with pKa=7.5)
- 0.01 M $MgCl_2$
- 0.02% Sodium azide (as a preservative)

Storage

Store reconstituted vials at 0-5 °C. Freezing and thawing of solutions is not recommended. When stored at 0-5 °C, this reagent as reconstituted is stable at least one month. Some minor insolubles may develop over time. These may be filtered or centrifuged out without adversely affecting the integrity of the reagent or assay results.

Procedure

The following assay is based on the binding of the dye HABA to avidin and the ability of biotin to displace the dye in stoichiometric proportions. This displacement of dye is accompanied by a change in absorbance at A_{500} which has a known extinction coefficient.

Sample Concentration

Approx. 1 mg protein/ml for biotinylated proteins of mol. wt. approx. 100,000 and with approx. 5 moles biotin/mole protein. Otherwise, samples should contain approx. 0.08 µmole/ml biotin. This should result in a change in absorbance at A_{500} of 0.1-0.4. If a change greater than this is observed, the sample should be diluted as this indicates there is more biotin present than avidin, and therefore dye displacement is no longer being measured. Also, samples containing glycerol must be dialyzed or desalted before use as the glycerol interferes with this assay.

In a 1 ml cuvette, pipet 900 µl HABA/avidin reagent (reconstituted as above). Read A_{500}. Add 100 µl sample, mix by inversion, then read A_{500}. In some cases, as with a biotinylated protein, the absorbance may slowly decrease with time. If this occurs, a wait of 2 minutes before reading the absorbance is recommended.

For colored samples, a blank must also be prepared as well. Dilute 100 µl sample with 900 µl water or diluent. Read A_{500}.

Results

Calculations

a) $\Delta A_{500} = 0.9(A^{\text{HABA/Avidin}}_{500}) - A^{\text{HABA/Avidin + sample}}_{500}$

0.9 = Dilution factor of HABA/Avidin upon addition of sample

Note: For colored samples, the absorbance of the blank must be taken into consideration as well:

$\Delta A_{500} = 0.9(A^{\text{HABA/Avidin}}_{500}) + A^{\text{sample blank}}_{500} - A^{\text{HABA/Avidin + sample}}_{500}$

0.9 = Dilution factor of HABA/Avidin upon addition of sample

b) µmole biotin/ml = $(\Delta A_{500}/34)(10)$

34 = mM extinction coefficient at 500 nm
10 = dilution factor of sample into cuvette

c) mole biotin/mole protein =

$$\frac{\text{µmole biotin/ml sample}}{\text{µmole protein/ml sample}}$$

Reference

1. Green, N. M., Methods Enzymol., Vol. 18A, 418 (1970).

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