

Avidin - ATTO 532 Assay Kit

Detection of Biotinylated Samples

| Cat.-No. | Amount |
|------------|------------|
| FP-304-532 | 100 assays |

For research use only!

Kit Contents

- **Avidin - ATTO 532**
1 vial containing 1 mg (c = 1 mg/ml in binding buffer)
- **Binding Buffer**
2 vials containing 25 ml each

Storage and Stability

Upon receipt, store the kit at -20°C. We recommend dividing the labeled avidin into aliquots and freeze at -20°C. Avoid freeze/thaw cycles!

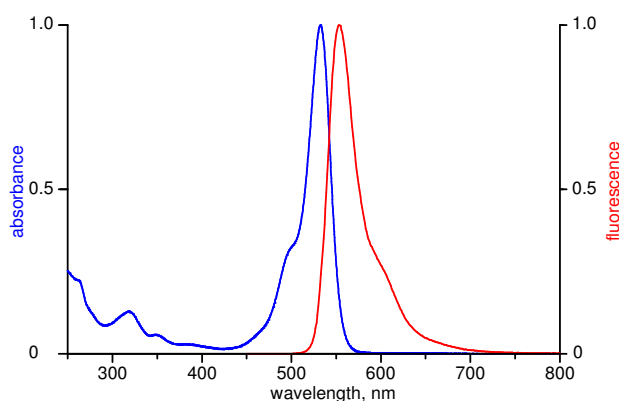
If stored as recommended, Jena Bioscience guarantees optimal performance of this kit for 12 months.

Spectroscopic data of ATTO 532

Excitation maximum: $\lambda_{Ex} = 532 \text{ nm}$

Emission maximum: $\lambda_{Em} = 553 \text{ nm}$

Extinction coefficient: $\epsilon_{max} = 115,000 \text{ cm}^{-1} \text{ M}^{-1}$



excitation and emission spectrum of ATTO 532

Introduction

Binding of biotin by avidin or streptavidin is the strongest known biological interaction with a dissociation constant in the range of 10^{-15} M . This Kit

is perfectly suited for binding and fluorescent detection of immobilized biotinylated samples such as proteins and DNA.

Free biotinylated samples can also be detected using this kit, but need to be separated from unbound fluorescent avidin after binding to obtain the accurate fluorescence signal.

Protocol

General notes

We recommend using 10 μl labeled avidin for 1 μg of biotinylated ds-DNA or protein, respectively.

If necessary, adjust the amount of avidin for your assay. The table below may give you a rough orientation, however, a specific adaptation might be required depending on your biotinylated sample and intended application.

In case you need to detect very low amounts of sample, dilute the labeled avidin, so that the required volume can be handled.

| biotinylated sample | | | fluorescent probe | |
|---------------------|---------|----------|-------------------|------------------|
| amount | size | moles | moles | volume |
| <i>ds-DNA</i> | | | <i>avidin</i> | |
| 1 μg | 50 bp | 30 pmol | 300 pmol | 20 μl |
| 1 μg | 200 bp | 7.5 pmol | 75 pmol | 5 μl |
| 1 μg | 1000 bp | 1.5 pmol | 15 pmol | 1 μl |
| <i>protein</i> | | | <i>avidin</i> | |
| 1 μg | 20 kDa | 50 pmol | 500 pmol | 30 μl |
| 1 μg | 60 kDa | 15 pmol | 150 pmol | 10 μl |
| 1 μg | 120 kDa | 8 pmol | 80 pmol | 5 μl |

Required volume of labeled avidin for different sized DNA and protein

Experimental protocol for immobilized samples

1. If necessary, dilute the labeled avidin using binding buffer.
2. Add the labeled avidin to your biotinylated sample and shake for 30 minutes at room temperature.
3. Wash your sample three times with binding buffer to remove excess free avidin.
4. Read out the fluorescence signal.

For further information and related products, please visit us at: www.jenabioscience.com