

**Avidin and Biotinylated Antibody Kit
(Rabbit anti-Goat IgG Kit)
(Cat. No.: BAK-008)**

Kit Composition

The Rabbit anti-Goat IgG Kit (BAK-008) contains 2ml biotinylated Rabbit anti-Goat IgG and 1mg each of the following: Purified Avidin (egg white), Avidin FITC (fluorescein isothiocyanate), Avidin TRITC (tetramethylrhodamine isothiocyanate), Avidin Horseradish Peroxidase, Avidin Alkaline Phosphatase.

Specific Applications

See individual datasheets for References.

General Procedure for Biotin Labeled Antibody

The following is a general Procedure and Trouble-Shooting Guide. The information is provided only for your convenience. The success of your experiments are not guaranteed by EY Laboratories, Inc.

1. Wash and block tissue section or blot. EY Laboratories, Inc. recommends that 1% purified Bovine Serum Albumin (BSA) or defatted milk powder be used for blocking to prevent non-specific binding. Do not use serum products, they contain glycoproteins which may lead to high levels of non specific background. After blocking, rinse briefly with recommended Buffer.
2. Dilute **Biotin Labeled Antibody** to a concentration of 5-50 µg/ml using recommended Buffer. Incubate section or blot for 30-90 minutes at room temperature in a moist chamber. Slightly longer incubation times may be required if incubation is done at 2-8°C. Rinse 3 times, 5 minutes *each* time with recommended Buffer.
3. Dilute and incubate **Avidin Conjugate** according to manufacturer directions.

Notes: Inhibition of antibody binding may be accomplished by using one of two procedures:

- A. Before proceeding to **Step #3** incubate antibody treated section or blot with inhibitory carbohydrate for 30-60 minutes at room temperature. NOTE: Complete inhibition may not occur.
- B. Preincubate diluted **Biotin Labeled Antibody** with inhibitory carbohydrate for 30-60 minutes at room temperature before applying to section or blot.

TROUBLE SHOOTING GUIDE

Problem	Cause	Solution
Weak or no Staining	<ol style="list-style-type: none"> 1. Low concentration of antibody conjugate. 2. Low concentration of avidin conjugate. 3. Insufficient incubation time. 4. Inappropriate treatment of sample prior to labeling. 	Causes #1 - #4 <ol style="list-style-type: none"> a. Increase incubation time. b. Increase concentration of sample (on blot) antibody conjugate and/or avidin conjugate. a. Treat section or blot with a different blocking reagent.
High Background	<ol style="list-style-type: none"> 1. Antibody conjugate and/or avidin conjugate is too concentrated. 2. Insufficient washing. 3. Insufficient blocking. 4. Sample contains endogenous enzymatic activity. 	<ol style="list-style-type: none"> a. Decrease concentration of respective reagents. b. Shorten incubation times. a. Perform multiple washings and prolong washing time. a. Treat section or blot with a different blocking reagent. a. Determine if sample contains activity which would give background staining in the absence of the avidin conjugate.
Unexpected Staining	Multiple causes	<ol style="list-style-type: none"> a. Perform control reactions. b. Use other cytochemical technique to prove or disprove the findings.

Additional Products

In addition to more than 300 labeled lectins, EY Laboratories, Inc. also manufactures a large selection of carbohydrate gels for lectin purification, antibody gels for purification of primary antibodies, and a number of different protein/glycoprotein gels. For further information, please contact customer service at EY Laboratories, Inc.

Sample Only

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Avidin and Biotinylated Antibody Kit Product Information

EY Laboratories' **AF** series antibodies are prepared from antisera by using affinity chromatography. The finished products may contain a low percentage of denatured protein due to unfavorable conditions during elution of the antibodies from the affinity column. Some cross reactivity studies have been done, contact Technical Service to request specific information.

Affinity purified antibodies and their conjugates are specifically prepared for laboratories who are involved with basic or diagnostic research. Purified antibodies give the highest possible ratio of the conjugate/antibody. This is an important factor for quantitative analysis of antigens. EY Laboratories' conjugated antibodies are designed for use in immunodiffusion, immunoelectrophoresis, fluorescent microscopy, light and electron microscopy.

The technology used in preparing the antigen specific and affinity purified antibody minimizes interference from other complex forming components in the antisera. Bovine serum albumin is used to coat vials containing affinity purified antibodies or their conjugates. This is to prevent loss of the antibody through adherence to the glass surface.

Catalog Number: BAF-013-2

Description: Biotin Conjugated Rabbit Affinity Purified Antibody to Goat IgG, 2mL

Lot Number:

Expiration Date: 1 year from date of manufacture

Protein Concentration:
(Based on OD280)

Chemical Used for Conjugation:
(where applicable) **Biotin**

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide is added as a preservative EXCEPT for peroxidase conjugates and alkaline phosphatase conjugates.

Storage: Store liquid frozen in aliquots EXCEPT for Ferritin and Alkaline Phosphatase conjugates which must be refrigerated, not frozen. Alkaline Phosphatase conjugates contain up to 50% glycerol.

Stability: The liquid material is stable for several years when stored in aliquots with 0.05% sodium azide added as a preservative.

NOTE: DO NOT add sodium azide to peroxidase conjugates.
Usage: Dilute 1% BSA in PBS at least 100 x before use.

Caution: Refer to the enclosed MSDS for information regarding affinity purified antibodies and their conjugates. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

For Research and Laboratory Use Only.

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Catalog Number: BA-000-1

Description: Pure Avidin (Egg White)

Lot Number:

Protein Concentration:
(Based on OD 280) 1 mg pure Avidin / vial. Reconstitute with Buffer.

Purification Procedure: Ammonium sulfate fractionation, gel filtration, followed by passage through a sterilizing filter.

Specificity: One mole of Avidin (MW 67,000) binds 4 moles of Biotin (MW 244.3). The Biotin-Avidin complex has a binding constant of 10^{15} .

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Storage: Store lyophilized powder refrigerated at 5-8°C or frozen. Store liquid frozen in aliquots. Avoid freeze-thaw cycles. Clarify by centrifugation.

Stability: The lyophilized material is stable for several years when stored frozen. After reconstitution the material is stable for at least 1 year when stored frozen in aliquots with 0.05% sodium azide added as a preservative.

Caution: Refer to the enclosed MSDS for information regarding Avidin. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Remarks: Avidin alone will denature at 85°C but is stable up to 132°C in the presence of Biotin. The binding is unaffected over a wide range of pH (2-13) and by chaotropic solvents such as guanidium chloride up to 3M.

References:

1. Donovan, J.W., et al., *Biochem.*, **12**, (1973), 512.
2. Chignell, C.F., et al., *J. Biol. Chem.*, **250**, (1975), 5622.
3. Orr, G.A., *J. Biol. Chem.*, **256**, (1981), 761.

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Avidin and Biotinylated Antibody Kit Product Information

Catalog Number: BA-101-1

Description: Avidin (egg white) - FITC

Lot Number:

Protein Concentration: 1 mg pure Avidin-FITC / 1 ml of Buffer.
(Based on OD 280)

Purification Procedure: Gel filtration performed after conjugation to remove free FITC.

Specificity: One mole of Avidin (MW 67,000) binds 4 moles of Biotin (MW 244.3). The Biotin-Avidin complex has a binding constant of 10^{15} .

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative.

Chemical Used for Conjugation: Fluorescein Isothiocyanate, FITC.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation.

Stability: The liquid material is stable for several years when stored frozen in aliquots with 0.05% sodium azide added as a preservative.

Caution: Refer to the enclosed MSDS for information regarding Avidin. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Remarks: Fluorescent Conjugates are extremely light sensitive.

References:

1. Heggeness, M.H., et. al., *Use of the Avidin-biotin complex for the localization of actin and myosin with Fluorescence Microscopy*. J. Cell Biol., **73**, (1977), 783.

Catalog Number: BA-102-1

Description: Avidin (egg white) - Rhodamine (TRITC)

Lot Number:

Protein Concentration: 1 mg pure Avidin-Rhodamine (TRITC) / 1 ml Buffer.
(Based on OD 280)

Purification Procedure: Gel filtration performed after conjugation to remove free rhodamine.

Specificity: One mole of Avidin (MW 67,000) binds 4 moles of Biotin (MW 244.3). The Biotin-Avidin complex has a binding constant of 10^{15} .

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contains 0.05% sodium azide as a preservative.

Chemical Used for Conjugation: Tetramethylrhodamine Isothiocyanate (TRITC).

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation.
Fluorescent Conjugates are extremely light sensitive.

Stability: The liquid material is stable for several years when stored frozen in aliquots with 0.05% sodium azide added as a preservative.

Caution: Refer to the enclosed MSDS for information regarding Avidin. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

Remarks: Fluorescent Conjugates are extremely light sensitive.

References:

1. Heggeness, M.H., et. al., *Use of the Avidin-Biotin Complex for the Localization of Actin and Myosin with Fluorescence Microscopy*, J. Cell Biol., **73**, (1977), 783.

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Avidin and Biotinylated Antibody Kit Product Information

Catalog Number: BA-104-1

Description: Avidin (egg white) - Horseradish Peroxidase

Lot Number:

**Protein Concentration:
(Based on OD 280)** 1 mg pure Avidin-Horseradish Peroxidase / 1 ml of Buffer.

Purification Procedure: Affinity Chromatography performed after conjugation.

Specificity: One mole of Avidin (MW 67,000) binds 4 moles of Biotin (MW 244.3). The Biotin-Avidin complex has a binding constant of 10^{15} .

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4.

Chemical Used for Conjugation: Horseradish Peroxidase.

Storage: Store liquid material frozen in aliquots in amber vials or covered with foil. Avoid freeze thaw cycles. Clarify by centrifugation. No preservatives have been added. Sodium azide will inactivate the enzyme, peroxidase.

Stability: The liquid material is stable for at least one year when stored frozen in aliquots

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

References:

1. Hsa, S.M., et. al., *Use Of Avidin-Biotin-Peroxidase Complex (ABC) In Immuno Peroxidase Techniques: A Comparison Between ABC And Unlabeled Antibody (PAP) Procedures.*, (1981), J. Histochem. Cytochem., **29** : 577.
2. Guesdon, J.L et. al., *The Use of Avidin-Biotin Interaction In Immuno Enzymatic Techniques*, (1979), J. Histochem. Cytochem., **27** : 1131.

Catalog Number: BA-105-1

Description: Avidin (egg white) - Alkaline Phosphatase

Lot Number:

**Protein Concentration:
(Based on OD 280)** 1 mg pure Avidin-Alkaline Phosphatase / 1 ml of Buffer.

Specificity: One mole of Avidin (MW 67,000) binds 4 moles of Biotin (MW 244.3). The Biotin-Avidin complex has a binding constant of 10^{15} .

Buffer: 0.01M Phosphate - 0.15M NaCl, pH 7.2 - 7.4. Contain 0.05% sodium azide as a preservative.

Chemical Used for Conjugation: Alkaline Phosphatase.

Storage: Store liquid refrigerated at 5-8°C in aliquots. DO NOT FREEZE! (20-50% Glycerol has been added to prevent freezing)

Stability: The liquid material is stable for at least 1 year when stored refrigerated in aliquots with 0.05% sodium azide added as a preservative.

Caution: Refer to the enclosed MSDS for information regarding lectins. The aluminum seals have sharp edges and the vial itself may have cracks which can cause lacerations. Use caution when opening the vial.

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MATERIAL SAFETY DATA SHEET

Effective Date: March 31, 2006

Revision 5

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PRODUCT IDENTIFICATION

Name: Purified proteins and enzymes labeled with D-Biotin.
 Catalog Number(s): BAP-01, BA-1102 to BA-9000, BAF-001 to BAF-2354, BAL-1104 to BAL-4701, BA-01 to BA-013, BA-108, BA-109, BA-111, BA-118, BA-119, BA-120, BA-121, BAT-2100 to BAT-2701.
 Formula: Complex polypeptides labeled with D-Biotin
 Synonyms: Protein A, Lectins, Secondary and Monoclonal Antibodies, Horseradish Peroxidase, Alkaline Phosphatase, Lactoperoxidase, Ferritin, and Urease labeled with D-Biotin.
 NOTE: D-Biotin is also known as vitamin H.

EMERGENCY INFORMATION

EY Laboratories, Inc.
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**EMERGENCY PHONE:
 650-342-3296**

HAZARDOUS COMPONENTS

Specific protein (s) as listed on the vial label. Solutions are at a concentration generally greater than 0.5mg protein/ ml. Powders are >>95% pure protein unless otherwise indicated on the vial label. Biological activity of these labeled proteins will vary. Vitamin H is an essential vitamin, required in very low amounts. The concentration of bound biotin is less than 10% of the protein amount (w/w). All solutions contain less than 0.05% sodium azide as a preservative.

HEALTH HAZARD INFORMATION

EXPOSURE LIMITS: None established. The toxicological properties of these products have not been thoroughly investigated. Care should be taken when handling any of these materials.
 EFFECTS OF OVEREXPOSURE: May cause localized eye, skin, or mucous membrane irritation. Some sensitive individuals may develop a chronic allergic reaction with exposure. The known effects are due to the protein.
 ROUTES OF EXPOSURE: Inhalation of powders and skin contact with liquids are the primary routes of exposure. Care should be taken to avoid the formation of aerosols when handling any of the solutions.

PHYSICAL CHARACTERISTICS

APPEARANCE: Powders are white to brown. Solutions will be clear to dark brown or red.
 SOLUBILITY: Powders are completely soluble in many biological buffers and water. All liquids are completely miscible in water and biological buffers.

FIRE AND EXPLOSION HAZARDS

EXTINGUISHING MEDIA: Not considered to be a fire hazard.
 Water spray or CO₂.
 SPECIAL FIRE FIGHTING NOTE: None required.
 NOTE: Most solutions contain less than 0.05% sodium azide as a preservative. Azide may react with lead and copper plumbing to form explosive metal azides. Flush with copious amounts of water when disposing material in the sink.

REACTIVITY DATA

STABILITY: Stable. Decomposition products are not known to be hazardous.
 HAZARDOUS POLYMERIZATION: Will NOT occur.
 INCOMPATIBILITY: None known. (Lead and copper may react with sodium azide).

SPILL / LEAK PROCEDURES

MATERIAL RELEASE / SPILL: Avoid contact with powder or liquid. Clean up spill with a paper towel soaked in household bleach. Do not allow solutions to dry on environmental surfaces. Wash affected area with detergent after the area has been treated with bleach.
 WASTE DISPOSAL: Incinerate, autoclave, or dispose of paper waste in accordance with all Local, State, and Federal regulations. Due to the small quantities of material involved these products are generally not considered to be environmental hazards. All of these proteins are fully biodegradable.

EMERGENCY FIRST AID PROCEDURES

May be harmful if swallowed, inhaled, or allowed to absorb through the skin. Wash contacted area with water for 15 minutes. If inhaled remove to fresh air. Report exposure to the appropriate safety official. Consult a physician if irritation occurs or if there is any indication of an allergic response, such as watering eyes, sneezing, or difficulty breathing.

SPECIAL HANDLING PRECAUTIONS

VENTILATION: No special ventilation is required but it is recommended to handle these reagents in a fume hood when possible.
 EYE PROTECTION: Not required under most circumstances but recommended as a safety precaution.
 RESPIRATORY PROTECTION: Recommended as a safety precaution, specifically when working with powders. An approved respirator may be required for those individuals already known to be sensitive to these materials.
 PROTECTIVE GLOVES: Required when handling any of these materials.

SPECIAL PRECAUTIONS

This material is for research and experimental application only. It is not intended for food, drug, household, agricultural, or cosmetic use. All materials should be handled only by technically qualified individuals experienced with working with potentially hazardous chemicals. The above information is correct to the best of our knowledge. The user should make independent decisions regarding completeness of the information, based on all sources available. EY Laboratories, Inc. shall not be held liable for any damage resulting from handling or contact with the above product.

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