For life science research only.
Not for use in diagnostic procedures.

Anti-Fluorescein, Fab fragments
from sheep

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For the detection of fluorescein-labeled compounds.

Cat. No. 11 426 338 910	Anti-Fluorescein-AP, Fab fragments 150 U, 200 µl
Cat. No. 11 426 346 910	Anti-Fluorescein-POD, Fab fragments 150 U

Store the conjugate at +2 to +8°C.

1. General Information

1.1. Contents

Label	Function / description	Content
Anti-Fluorescein-AP, Fab fragments	Solution, stabilized	1 vial, 200 µl
Anti-Fluorescein-POD, Fab fragments	Lyophilized, stabilized	1 vial, 150 U

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the conjugate is stable through the expiry date printed on the label.

Label	Storage
Anti-Fluorescein-AP, Fab fragments	Store at +2 to +8°C.
Anti-Fluorescein-POD, Fab fragments	

Reconstitution

Anti-Fluorescein-POD, Fab fragments

Dissolve the lyophilizate in 1 ml double-distilled water; this results in a concentration of 150 U/ml.

1.3. Additional Equipment and Reagent required

For preparation of Anti-Fluorescein-AP working solution

- 100 mM Tris-HCl*, 150 mM NaCl, pH 7.5
- Blocking Reagent* or dry milk powder, or fetal calf serum, or normal sheep serum

For preparing substrate solutions

See section, **General Considerations***, for information on preparing solutions.*

- NBT*
- BCIP*
- Fast Red, TR-salt/NABP
- CSPD*
- DAB Substrate*
- Tris-HCl*
- AEC (3-amino-9-ethyl carbazole)
- CN (4-chloro-1-naphtol)
- pNPP (4-nitrophenyl phosphate)
- ABTS*

1.4. Application

Use the antibody conjugates for the detection of fluorescein-labeled compounds, such as fluorescein-labeled nucleic acids, lectins, and antibodies. The conjugates are only applicable for:

- Immunoblotting
- Histochemistry
- ELISA

using an anti-mouse Ig fluorescein, $F(ab)_2$-fragment.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Substrate solutions for alkaline phosphatase	Preparation/Composition
NBT*/BCIP* (5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium chloride)	▪ 0.38 mM BCIP, 0.41 mM NBT in 200 mM Tris-HCl, pH 9.5, 10 mM $MgCl_2$. ▪ Dissolve BCIP and NBT in 70% dimethylformamide (v/v). ▪ The resulting product is blue and insoluble in water, but soluble in ethanol. *A ready-to-use 10x stock solution is also available*.*
Fast Red, TR-salt/NABP (4-chloro-2-methyl-benzene/diazonium chloride/ naphthol AS-BI-phosphate)	▪ 38.8 mM Fast Red, TR-salt, 0.4 mM NABP in 100 mM Tris-HCl, pH 9.8. ▪ Dissolve NABP in dimethylsulfoxide; add Fast Red shortly before use. ▪ The resulting product is red, insoluble in water, but soluble in ethanol.
CSPD* chemiluminescent substrate (Disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2′-(5′-chloro) tricyclo [3.3.1.13,7]decan}-4-yl) phenyl phosphate	–
Substrate solutions for peroxidase	**Preparation/Composition**
DAB* (Diaminobenzidine (3,4,3′,4′, tetraaminobiphenyl)	▪ 1.39 mM DAB, 0.01% H_2O_2 (v/v) in 50 mM Tris-HCl, pH 7.3. ▪ The formed product is brown, insoluble in water and ethanol.
AEC (3-amino-9-ethyl carbazole)	▪ 0.32 mM AEC, 0.002% H_2O_2 (v/v) in 50 mM Tris-HCl, pH 7.3. ▪ Dissolve AEC in dimethylsulfoxide. ▪ The resulting product is red, insoluble in water, but soluble in ethanol.
CN (4-chloro-1-naphtol)	▪ 5.6 mM CN, 0.01% H_2O_2 (v/v) in 50 mM Tris-HCl, pH 7.4, 150 mM NaCl. ▪ Dissolve CN in methanol. ▪ The resulting product is bluish black, insoluble in water, but soluble in ethanol.
Substrate solutions with water-soluble products for ELISA for alkaline phosphatase	**Preparation/Composition**
pNPP (4-nitrophenyl phosphate)	▪ 10 mM pNPP in 1 M diethanolamine buffer, pH 9.8, 0.5 mM $MgCl_2$. ▪ The formed product is yellow and soluble in water. ▪ Measurement at 405 nm.
Substrate solutions with water-soluble products for ELISA for peroxidase	**Preparation/Composition**
ABTS* 2,2′ Azino-di-(3-ethylbenzthiazoline sulfonate [6])	▪ 100 mg ABTS substrate in 3.25 mM sodium perborate, 39.8 mM citric acid, 60 mM disodium hydrogen phosphate, pH 4.4 to 4.5. ▪ The formed product is green and soluble in water. ▪ Measurement at 405 nm.

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Wash hands thoroughly after handling samples and reagents.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Anti-Fluorescein-AP, Fab fragments

1. Dilute the solution in 100 mM Tris-HCl*, 150 mM NaCl, pH 7.5.

2. If necessary, 1% Blocking Reagent* (w/v) or dry milk powder, or 1 to 5% heat-inactivated fetal calf serum (v/v) or sheep normal serum can be added to the conjugate dilution buffer for reduction of nonspecific binding.

Anti-Fluorescein-POD, Fab fragments

1. After reconstitution of the lyophilizate, dilute the solution in 100 mM Tris-HCl*, 150 mM NaCl, pH 7.5.

2. If necessary, 1% Blocking Reagent* (w/v) or dry milk powder, or 1 to 5% heat-inactivated fetal calf serum (v/v) or sheep normal serum can be added to the conjugate dilution buffer for reduction of nonspecific binding.

2.2. Parameters

Working Concentration

Preparation of antibody dilutions

The working concentration of antibody depends on the application. The following concentrations should be taken as a guideline:

Application	Dilution Anti-Fluorescein-AP	Dilution Anti-Fluorescein-POD
Southern blots and dot blots Detection of fluorescein-labeled nucleic acids on membranes.	▪ 1:5,000 = 150 mU/ml ▪ For 500 blots.	▪ 1:1,000 = 150 mU/ml ▪ For 500 blots.
(*In situ* hybridization) Detection of fluorescein-labeled nucleic acids in cells and tissues.	▪ 1:100 – 1:500 = 7.5 U/ml – 1.5 U/ml ▪ For 400 – 2,000 hybridizations.	▪ 1:20 – 1:100 = 7.5 U/ml – 1.5 U/ml ▪ For 400 – 2,000 hybridizations.
Detection of fluorescein-labeled sugars in glycoconjugates.	▪ 1:1,000 = 750 mU/ml ▪ For 20 blots.	▪ 1:200 = 750 mU/ml ▪ For 20 blots.
Western blotting	▪ 1:1,500 – 1:3,000 = 500 mU/ml – 250 mU/ml ▪ For 30 – 60 blots.	▪ 1:150 – 1:300 = 1,000 mU/ml – 500 mU/ml ▪ For 30 – 60 blots.
Immunohistochemistry	▪ 1:1,500 – 1:3,000 = 500 mU/ml – 250 mU/ml ▪ For 6,000 – 12,000 sections.	▪ 1:300 – 1:600 = 500 mU/ml – 250 mU/ml ▪ For 6,000 – 12,000 sections.
ELISA	▪ 1:2,500 – 1:5,000 = 300 mU/ml – 150 mU/ml ▪ For 2,500 – 5,000 tests.	▪ 1:1,000 – 1:3,000 = 150 mU/ml – 50 mU/ml ▪ For 5,000 – 15,000 tests.

3. Additional Information on this Product

3.1. Test Principle

Anti-Fluorescein-AP, Fab fragments

The reagent is an anti-fluorescein antibody, Fab fragments from sheep, conjugated with alkaline phosphatase (AP).

① After immunization with fluorescein, the sheep IgG was purified by ion exchange chromatography and the specific IgG was isolated by immunosorption.

② The Fab fragments obtained by papain digestion were purified by gel filtration, conjugated with AP, and stabilized in 50 mM triethanolamine buffer, 3 M NaCl, 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 1% bovine serum albumin* (w/v), pH 7.6.

Anti-Fluorescein-POD, Fab fragments

The reagent is an anti-fluorescein antibody, Fab fragments from sheep, conjugated with horseradish peroxidase (POD).

① After immunization with fluorescein, the sheep IgG was purified by ion exchange chromatography and the specific IgG was isolated by immunosorption.

② The Fab fragments obtained by papain digestion were purified by gel filtration, conjugated with POD, and stabilized in 60 mM Tris-HEPES buffer, 0.4% bovine immunoglobulin (w/v), 0.01% MIT (w/v), pH 7.2.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.
Update to include new safety Information to ensure handling according controlled conditions.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Blocking Reagent	50 g	11 096 176 001
Tris hydrochloride	500 g	10 812 846 001
BCIP	250 mg	10 760 994 001
	1 g	11 585 002 001
NBT	5 g	11 585 029 001
DAB Substrate	1 pack	11 718 096 001
CSPD, ready-to-use	2 x 50 ml	11 755 633 001
ABTS Tablets and Buffer Sets	ABTS Tablets, 5 mg	11 204 521 001
	ABTS Buffer, 125 ml	11 204 530 001
	ABTS Buffer, 16.7 g for 1 l	11 112 597 001
	ABTS Tablets, 50 mg	11 112 422 001

4.4. Trademarks

ABTS is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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