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Not for use in diagnostic procedures.

Anti-Fluorescein
from mouse IgG_1 (clone B13-DE1)

Version: 17
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Monoclonal antibody to fluorescein from mouse-mouse hybrid cells.

Cat. No. 11 426 320 001 100 µg

Store the product at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Anti-Fluorescein	Lyophilized immunoglobulin, stabilized.	1 vial, 100 µg

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / Bottle	Label	Storage
1	Anti-Fluorescein	Store at +2 to +8°C.

Storage Conditions (Working Solution)

The reconstituted antibody solution is stable for 6 months at +2 to +8°C.
The solution can be aliquoted and stored at −15 to −25°C.
⚠ ***Avoid repeated freezing and thawing.***

Coating of microplates and sample dilution

Coating of microplates and sample dilution		
Solution	**Storage/Stability**	**Use**
Dilution buffer	2 months at +2 to +8°C ⓘ *Only if POD-inactivating preservation agents (like thymol) is used with Anti-Fluorescein-POD, and similarly no sodium azide is used with Anti-Fluorescein-AP.*	Dilution of antibodies.
Coating stock solution	12 months at −15 to −25°C ⚠ ***Aliquot this stock solution and store at −15 to −25°C°***	For preparation of Coating working solution.
Coating working solution	2 days at +2 to +8°C.	Coating microplates.
Blocking solution	4 weeks at +2 to +8°C	Blocking unspecific binding on microplates.
Wash buffer	1 week at +2 to +8°C	Washing after sample and antibody application to microplates.
Detection with Anti-Fluorescein-POD, Fab fragments		
Solution	**Storage/Stability**	**Use**
Sample solution	2 days at +2 to +8°C	Dilution of samples.
Anti-Fluorescein-POD, Fab fragments	2 weeks at +2 to +8°C.	Detection of fluorescein-labeled compounds.
Substrate buffer	At +2 to +8°C.	To prepare substrate solution.
Substrate solution	3 month at +2 to +8°C ⚠ ***Store protected from light!***	For color development.

Detection with Anti-Fluorescein-AP, Fab fragments		
Solution	**Storage/Stability**	**Use**
Anti-Fluorescein-AP, Fab fragments	2 weeks at +2 to +8°C	Detection of fluorescein-labeled compounds.
Substrate buffer	At +2 to +8°C	To prepare substrate solution.
Substrate solution	3 days at +2 to +8°C	For color development.

Reconstitution

Dissolve the lyophilizate in 1 ml double-distilled water, to a concentration of 0.1 mg antibody/ml.

1.3. Additional Equipment and Reagent required

Coating of microplates

- Blocking Reagent*
- Tris-Hydrochloride*
- PBS* (phosphate buffered saline)
- Double-distilled water
- Tween 20*
- Sodium chloride (NaCl), A.R.
- Sodium azide

  ⚠ ***Do not add sodium azide when using fluorescein-POD.***

Detection with Anti-Fluorescein-POD, Fab fragments

See section, **Working Solution** *for information on preparing solutions.*

- Anti-Fluorescein POD, Fab fragments*
- Dilution buffer
- ABTS Solution*

  The ABTS Solution is ready-to-use. There is no need to prepare the Substrate buffer and Substrate Solution.
- Sodium perborate trihydrate ($NaBO_2 \times H_2O_2 \times 3\ H_2O$), A.R.
- Citric acid monohydrate ($C_3H_8O_7 \times H_2O$), A.R.
- Di-sodium hydrogen phosphate ($Na_2HPO_4 \times H_2O$), A.R.

Detection with Anti-Fluorescein-AP, Fab fragments:

See section, **Working Solution** *for information on preparing solutions.*

- Anti-Fluorescein AP, Fab fragments*
- Dilution buffer
- Diethanolamine
- $MgCl_2$
- 4-Nitrophenyl phosphate

1.4. Application

Anti-Fluorescein is suitable for the detection of fluorescein-labeled compounds, such as fluorescein-labeled proteins and nucleic acids in various test systems:

- *In situ* hybridization
- Western blot
- ELISA
- Immunohistocytochemistry

The detection of bound antibody can be carried out directly in one step using an anti-mouse Ig fluorochrome/enzyme conjugate, or in a two-step procedure with anti-mouse Ig fluorescein and, subsequently, anti-fluorescein enzyme conjugate.

The antibody contains no protein and can hence be used for coating and labeling.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Preparation of sample material

- For the ELISA, use nucleic acids into which Fluorescein-12-dUTP* has been incorporated by either random priming or nick translation, in a concentration of 5 to 50 ng/ml; apply in at least 5 concentrations.
- Use proteins labeled with 5(6) carboxyfluorescein N-hydroxysuccinimide ester (FLUOS) in a concentration of 0.5 to 10 ng/ml; apply in at least 5 concentrations.

General Considerations

Microplates

For quantitative tests, the quality of the microplates is a critical parameter.
- Use plates which exhibit high binding capacity for peptides/proteins, also in the presence of detergents.
- The microplates should also exhibit a great homogeneity within and among the plates; this should be proved by the manufacturer.
- Independent of the suitability of the plate material (plastic composition, surface quality, γ-irradiation) for enzyme immunoassays, plates of different manufacturers and even plates of the same manufacturers but of different batches can produce different results.

Pipetting

Make sure that when pipetting sample, antibody, and conjugate solutions, the volumes used are never greater than the volume of coating solution.
This prevents contact with uncoated surfaces.

Evaluation

Plot the measured absorbance values on the ordinate against the concentration values on the abscissa on semi-logarithmic graph paper.

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

Coating of microplates and sample dilution

Solution	Preparation/ Composition	Storage and Stability	For use in...
Coating stock solution	Dissolve 100 µg Anti-Fluorescein in 1 ml double-distilled water to give a concentration of 0.1 mg/ml.	Aliquot and store 12 months at −15 to −25°C.	Coating working solution
Coating working solution	Dilute the Coating stock solution with PBS*, pH 7.4. *Working concentration: 2 µg/ml, depending on the plate material.*	Store 2 days at +2 to +8°C.	Microplate coating
Blocking solution	1% Blocking Reagent* (w/v), in 40 mM Tris-HCl*, pH 7.4, 150 mM NaCl, and 0.1% sodium azide (w/v). ***When using Fluorescein-POD, do not add sodium azide.***	Store 4 weeks at +2 to +8°C.	Blocking
Wash buffer	40 mM Tris-HCl*, pH 7.4, 150 mM NaCl with 0.1% Tween 20* (v/v).	Store 1 week at +2 to +8°C.	Washing
Dilution buffer	1% Blocking Reagent* (w/v) in 40 mM Tris-HCl*, pH 7.4, 150 mM NaCl	Store 2 months at +2 to +8°C when no POD-inactivating preservation agents, such as thymol, is used with anti-fluorescein-POD, and similarly no sodium azide is used with anti-fluorescein-AP.	Sample solution
Sample solution	Dilute samples in Dilution buffer.	Store 2 days at +2 to +8°C.	Dilute samples

Detection with Anti-Fluorescein-POD, Fab fragments

Solution	Preparation/ Composition	Storage and Stability	For use in...
Anti-Fluorescein-POD, Fab fragments	Reconstitute accordingly to the Instructions for Use. ▪ Working solution in Dilution buffer. ▪ Recommended concentration range: 100 to 250 U/ml.	Store 2 weeks at +2 to +8°C.	Detection
Substrate buffer	3.25 mM sodium perborate, 39.8 mM citric acid, 60 mM disodium hydrogen phosphate, pH 4.4 to 4.5.	Store at +2 to +8°C.	Substrate solution
Substrate solution	Dissolve 100 mg ABTS Substrate* in 100 ml Substrate buffer.	Store 3 months at +2 to +8°C. ***Keep protected from light.***	Detection

Detection with Anti-Fluorescein-AP, Fab fragments

Solution	Preparation/ Composition	Storage and Stability	For use in...
Anti-Fluorescein-AP, Fab fragments	Reconstitute accordingly to the Instructions for Use. ▪ Working solution in Dilution buffer. ▪ Working concentration range: 100 to 250 mU/ml.	Store 2 weeks at +2 to +8°C.	Detection
Substrate buffer	1 M Diethanolamine, 0.5 mM $MgCl_2$, pH 9.8.	Store at +2 to +8°C.	Substrate solution
Substrate solution	Dissolve 371 mg 4-Nitrophenyl phosphate × 6 H_2O in 100 ml Substrate buffer.	Store 3 days at +2 to +8°C.	Detection

2.2. Protocols

ELISA

See section, **Working Solution** *for additional information on preparing solutions.*
If required, the sensitivity and speed of the test can be varied by altering the quantities used, and the incubation times and temperatures.
Perform an entire series on the same microplate, if possible. 3-fold analysis is recommended for standards and samples.

1. Pipette 250 µl Coating solution into the wells of the microplate.
   – Cover the plate tightly and incubate for 1 hour at +37°C.
2. Remove the contents of the microplate onto blotting paper by suction or tapping.
3. Pipette 250 µl of Blocking solution into the wells and incubate for 15 minutes at +37°C.
4. Remove the contents of the microplate onto blotting paper by suction or tapping.
5. Pipette 200 µl of Sample solution into the wells and incubate for 1 hour at +37°C.
6. Remove the contents of the microplate onto blotting paper by suction or tapping.
7. Wash the wells at least 3 times with Wash buffer and carefully remove residual buffer.
8. Pipette either 200 µl Anti-Fluorescein-POD* or 200 µl Anti-Fluorescein-AP* into the wells.
9. Cover plate tightly and incubate for 1 hour at +37°C.
10. Remove the contents of the microplate onto blotting paper by suction or tapping.
11. Wash the wells at least 3 times with Wash buffer and carefully remove any residual buffer.
12. Pipette 200 µl Substrate solution (depending on the use of POD or AP) into the wells.
    – Incubate until the color development is strong enough for photometric evaluation.
13. Measure absorbance against corresponding Substrate solutions at 405 nm.

2.3. Parameters

Detection range

The exact determination of concentration at the lower detection limit is largely dependent upon the degree of accuracy obtained in the laboratory.

Specificity

The monoclonal antibody reacts with free and bound fluorescein.

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
(*In situ* hybridization) Detection of fluorescein-labeled nucleic acids on chromosomes.	▪ 1:250 – 1:500 = 0.2 – 0.4 µg/ml ▪ For 5,000 – 10,000 hybridizations.
Detection of fluorescein-labeled proteins by immunoblotting.	▪ 1:50 – 1:200 = 0.5 – 2 µg/ml ▪ For 4 – 20 blots of 10 ml incubation volume.
Detection of fluorescein-labeled proteins in immunohistochemistry.	▪ 1:50 – 1:200 = 0.5 – 2 µg/ml ▪ For 1,000 – 4,000 sections.
ELISA	▪ 1:25 – 1:50 = 2 – 4 µg/ml ▪ For 125 – 250 tests.

3. Additional Information on this Product

3.1. Test Principle

Principle

The following steps describes the application of Anti-Fluorescein in ELISA.
The degree of fluorescein-labeling can be determined quantitatively.

① Anti-Fluorescein monoclonal antibody is fixed by adsorption to the wall of a microplate.

② Remaining nonspecific binding sites on the wall are subsequently saturated with an appropriate blocking solution.

③ A correspondingly diluted fluorescein-labeled compound is added to the microplate and incubated.

④ Sample is removed from the microplate and the plate is washed.

⑤ The fluorescein-labeled compound that is bound to the capture-antibody is labeled with Anti-Fluorescein-POD, Fab fragments* or Anti-Fluorescein-AP, Fab fragments*.

⑥ Enzyme bound to this complex is then photometrically determined using a soluble substrate:
– ABTS Substrate* perborate system for peroxidase (POD), or
– 4-Nitrophenyl phosphate for alkaline phosphatase (AP).

Preparation

To obtain monoclonal antibodies, Balb/c mice were immunized with KLH-bound fluorescein and the spleen cells subsequently fused with myeloma cells of the cell line NS-1. The antibodies were purified by gel filtration, diluted in 10 M potassium phosphate buffer, 75 mM NaCl, 2% raffinose (w/v), 0.01% 2-methylisothiazolone (MIT) (w/v), pH 7.4 and subsequently lyophilized.

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	27 g, for one liter blocking solution, *Not available in US*	11 112 589 001
Fluorescein-12-dUTP	custom fill	11 375 601 103
Tris hydrochloride	500 g	10 812 846 001
Anti-Fluorescein, Fab fragments	Anti-Fluorescein-AP, Fab fragments, 150 U, 200 µl	11 426 338 910
	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
Anti-Fluorescein-POD, Fab fragments	Anti-Fluorescein-POD, Fab fragments, 150 U	11 426 346 910
ABTS Solution	3 x 100 ml, for 1,500 to 3,000 reactions	11 684 302 001

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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