

# Anti-Fluorescein

Monoclonal antibody to fluorescein from mouse-mouse hybrid cells (clone B13-DE1)

Cat. No. 11 426 320 001

100 µg

Version July 2005

Store at +2 to +8°C

## Product overview

**Formulation** Lyophilized immunoglobulin, stabilized.

**Antibody type** IgG1 subclass.

**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.

**Reconstitution** Dissolving the lyophilizate in 1 ml double distilled water results in a concentration of 0.1 mg antibody/ml.

**Working concentration**

Detection of fluorescein-labeled...	Dilution	Conc. [µg/ml]	Sufficient for...
nucleic acids on chromosomes ( <i>in situ</i> hybridization)	1:250 – 1:500	0.2 – 0.4	5,000 – 10,000 <i>in situ</i> hybridizations
proteins by immunoblotting	1:50 – 1:200	0.5 – 2	4 – 20 blots of 10 ml incubation volume
proteins in immunohistochemistry	1:50 – 1:200	0.5 – 2	1000 – 4000 sections
ELISA	1:25 – 1:50	2 – 4	125 – 250 tests

**Application** The antibody is suitable for the detection of fluorescein-labeled compounds *e.g.*, fluorescein-labeled proteins and nucleic acids in various test systems such as:

- *in situ* hybridization
- Western Blot
- ELISA
- Immunohisto/cytochemistry

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.

**Specificity** The antibody reacts specifically with free and bound fluorescein.

**Stability** The lyophilized antibody is stable at +2 to +8°C until the control date printed on the label.

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; repeated freezing and thawing should be avoided.

## Procedures and required material

### Introduction

#### General

The following procedure describes exemplary the application in ELISA. In this ELISA, the degree of fluorescein labeling can be determined quantitatively

#### Test principle

Please refer to the following table.

Step	Description
1	The monoclonal antibody anti-fluorescein is fixed by adsorption to the wall of a microplate
2	Remaining unspecific 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	The 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* or anti-fluorescein AP*.
6	The enzyme bound to this complex is then photometrically determined using a soluble substrate (ABTS <sup>1</sup> )* - perborate system for POD, or 4-nitrophenyl phosphate for AP).

### Before you begin

#### Additional reagents required

- ABTS\*
- Blocking Reagent\*
- Citric acid monohydrate (C<sub>3</sub>H<sub>8</sub>O<sub>7</sub> × H<sub>2</sub>O), A.R.
- Sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), anhydrous, A.R.
- Sodium chloride (NaCl), A.R.
- Sodium hydrogen carbonate (NaHCO<sub>3</sub>), A.R.
- Di-sodium hydrogen phosphate (Na<sub>2</sub>HPO<sub>4</sub> × H<sub>2</sub>O), A.R.
- Sodium perborate trihydrate (NaBO<sub>2</sub> × H<sub>2</sub>O<sub>2</sub> × 3 H<sub>2</sub>O), A.R.
- 4-Nitrophenyl phosphate
- Anti-fluorescein AP, Fab fragments\*
- Anti-fluorescein POD, Fab fragments\*
- Tris-hydrochloride\*
- Tween 20\*

#### Microplates

In this type of quantitative tests the quality of the microplates is a critical parameter. Thus, only plates should be used 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, which should be proved by the manufacturer.

**Preparation of working solutions**

Please refer to the following table

Solution	Preparation/Composition	Storage/Stability
Dilution buffer	1% Blocking Reagent (w/v) in 40 mM Tris-HCl, pH 7.4, 150 mM NaCl. <b>Note:</b> When using streptavidin-POD do not add sodium azide.	2 months at +2 to +8°C when no POD-inactivating preservation agents (e.g. thymol) is used with anti-fluorescein-POD, and similarly no sodium azide is used with anti-fluorescein-AP.
Coating stock solution	Dissolve 100 µg Anti-fluorescein in 1 ml double distilled water to give a concentration of 0.1 mg/ml. <b>Note:</b> Aliquot this stock solution and store at -15 to -25°C.	12 months at -15 to -25°C
Coating working solution	Dilute the stock solution with PBS (phosphate buffered saline), pH 7.4. The concentration used depends on the plate material. Recommended concentration: 2 µg/ml.	2 days at 2-8°C.
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). <b>Note:</b> When using streptavidin-POD do not add sodium azide.	4 weeks at +2 to +8°C
Wash buffer	40 mM Tris-HCl, pH 7.4, 150 mM NaCl with 0.1% Tween 20 (v/v).	1 week at +2 to +8°C
Sample solution	Dilute samples in Dilution buffer.	2 days at +2 to +8°C
<b>Detection with anti-fluorescein-POD, Fab fragments</b>		
anti-fluorescein-POD, Fab fragments	Reconstitution according to the working instruction. Working dilution in Dilution buffer. Recommended concentration range 100-250 mU/ml.	2 weeks at +2 to +8°C
Substrate buffer	3.25 mM Sodium perborate, 39.8 mM citric acid, 60 mM disodium hydrogen phosphate, pH 4.4 - 4.5.	at +2 to +8°C
Substrate solution	Dissolve 100 mg ABTS in 100 ml Substrate buffer.	3 month at +2 to +8°C, if protected from light
<b>Detection with anti-fluorescein-AP, Fab fragments</b>		
Anti-fluorescein-AP, Fab fragments	Reconstitution according to the working instruction. Working dilution in Dilution buffer. Recommended concentration range: 100 -250 mU/ml.	2 weeks at +2 to +8°C
Substrate buffer	1 M Diethanolamine, 0.5 mM MgCl <sub>2</sub> , pH 9.8.	at +2 to +8°C
Substrate solution	Dissolve 371 mg 4-Nitrophenyl phosphate × 6 H <sub>2</sub> O in 100 ml Substrate buffer.	3 days at +2 to +8°C

**Preparation of sample material**

For this ELISA nucleic acids into which fluorescein-12-dUTP\* has been incorporated either by random-priming (1) or nick translation (2), should have a concentration of 5 - 50 ng/ml and applied in at least 5 concentrations. Similarly, proteins labeled with 5(6) carboxyfluorescein N-hydroxysuccinimide ester (FLUOS) should have a concentration of 0.5 -10 ng/ml and should be applied in at least 5 concentrations.

**Microplates**

Independent of the suitability of the plate material (plastic composition, surface quality, γ-irradiation) for enzyme immunoassays, it is pointed out that 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.

**Standard protocol (Sandwich ELISA)**

**Protocol**

If required, the sensitivity and speed of the test can be varied by altering the quantities used, the incubation times and the temperatures.

A whole series should if possible be performed on then same microplate. 3-fold analysis are recommended for standards and samples.

Step	Action
1	• Pipette 250 µl Coating solution into the wells of the microplate. • Cover the plate tightly and incubate for 1 h 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 min 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 h 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 <b>or</b> • 200 µl anti-fluorescein-AP into the wells.
9	Cover plate tightly and incubate for 1 h 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 and incubate until the color development is strong enough for photometric evaluation.
13	Measure absorbance against corresponding Substrate solutions at 405 nm.

**Detection limit**

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

**References**

- 1 Feinberg, A.P. & Vogelstein, B. (1983) *Anal. Biochem.* **132**, 6-13.
- 2 Rigby, P.W.J. et al. (1977) *J. Mol. Biol.* **113**, 237-251.

## Related products

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Product	Pack size	Cat. No.
Fluorescein-12-dUTP	25 nmol (25 µl)	11 373 242 001
Fluorescein Protein Labeling Kit	1 kit	11 386 093 001
Anti-fluorescein-AP, Fab fragments	150 U	11 426 338 001
Anti-fluorescein-POD, Fab fragments	150 U	11 426 346 001
ABTS Solution	3 × 100 ml	11 684 302 001
Blocking Reagent	50 g	11 096 176 001
Tris-hydrochloride	500 g	10 812 846 001
Tween 20	5 × 10 ml	11 332 465 001
BM Blue POD Substrate	100 ml	11 484 281 001
BM Chemiluminescence ELISA Substrate (POD)	for 250 ml	11 582 950 001
AttoPhos Substrate Set	1 set	11 681 982 001

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\* available from Roche Applied Science  
ABTS is a Trademark of Roche.  
Tween is a Trademark of ICI Americas Inc., USA.

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