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

Anti-Digoxigenin
from mouse - mouse-hybrid cells (clone 1.71.256)

Version: 09
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For the detection of digoxigenin-labeled compounds.
Lyophilized, stabilized

Cat. No. 11 333 062 910 100 µg

Store the lyophilizate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Anti-Digoxigenin monoclonal antibody	1 vial, 100 µg

1.2. Storage and Stability

Storage Conditions (Product)

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

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

Reconstitution

Dissolve the lyophilizate in 1 ml double-distilled water; this results in a concentration of 0.1 mg antibody/ml.
The reconstituted antibody solution is stable for up to 6 months at +2 to +8°C. The solution can be stored in aliquots at −15 to −25°C.
⚠ ***Avoid repeated freezing and thawing.***

1.3. Additional Equipment and Reagent required

For ELISA

- ABTS Substrate, (2,2h-Azino-di-[3-ethylbenzthiazoline-sulfonate (6)])*
- Blocking Reagent*
- Anti-Digoxigenin-AP, Fab fragments*
- Anti-Digoxigenin-POD, Fab fragments*
- 4-Nitrophenyl phosphate

For Preparation of Additional Solutions

- Tris-HCl*
- Tween 20*
- Sodium chloride (NaCl), A. R.
- Citric acid, monohydrate ($C_6H_8O_7 \times H_2O$), A. R.
- Sodium carbonate (Na_2CO_3), anhydrous, A. R.
- Sodium hydrogen carbonate ($NaHCO_3$), A. R.
- Disodium hydrogen phosphate dihydrate ($Na_2HPO_4 \times 2\ H_2O$), A. R.
- Sodium perborate trihydrate ($NaBO_2 \times H_2O_2 \times 3\ H_2O$), A. R.

1.4. Application

The antibody is used for the detection of digoxigenin-labeled compounds, such as digoxigenin-labeled proteins and nucleic acids in different detection systems.
Applications include:

- *In situ* hybridizations
- Immunoblotting (western blotting)
- Immunohistochemistry
- ELISA

⚠ ***The antibody is not stabilized with protein and is therefore suitable for coating and labeling purposes.***

The detection of the bound antibody can be performed in 2 ways:

- Directly, in one step with anti-mouse Ig-fluorochrome/enzyme conjugate, or
- In two steps with anti-mouse Ig-digoxigenin and later with an anti-digoxigenin-fluorochrome/enzyme conjugate.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Microplates

For quantitative tests, the quality of the microplate is a critical parameter. Only use microplates that have high-binding capacity for peptides and proteins, and when in the presence of detergents. The plates must also exhibit homogeneity within and among the plates and proven by the manufacturer by, for example, γ-irradiated microplates. Depending on the suitability of the plates (plastic composition, surface quality, γ-irradiation) used for enzyme immunoassays, different results may be obtained when microplates from different manufacturers and even when different lots from the same manufacturer are used.

Pipetting

The volumes of sample, antibody, and conjugate solution must not exceed the volume of the coating solution in order to prevent contact with uncoated surfaces.

Assay Procedure

By modifying quantities, incubation times and temperatures, sensitivity and duration of the assay may be adjusted to individual requirements.

Detection Limit

The exact determination of concentrations at the lower detection limit depends on the accuracy obtained in the laboratory.

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

Preparation of Additional Solutions

Prepare the following solutions prior to beginning the ELISA assay.

Solution	Contents	Preparation	Storage and Stability
1	Coating buffer	PBS (phosphate buffered saline), pH 7.4	Store 6 months at +2 to +8°C.
2	Basic buffer	40 mM Tris-HCl, pH 7.4, 150 mM NaCl	Store 1 week at +2 to +8°C.
3	Sample, Standard, Conjugate, Antibody buffer	1% Blocking reagent (w/v) in Basic buffer (solution 2); using Anti-Digoxigenin-POD, Fab fragments. ⚠ ***Do not add sodium azide.***	Store 2 months at +2 to +8°C when: ▪ For the Anti-Digoxigenin-POD, Fab fragments, peroxidase-inactivating preservation agents, such as Thymol were not used. ▪ For the Anti-Digoxigenin-AP, Fab fragments, sodium azide was not used to avoid microbial infection.
4a	Coating solution	Dissolve Anti-Digoxigenin in double-distilled water to a concentration of 1 mg/ml.	Store aliquots 12 months at -15 to -25°C.
4b		▪ Dilute stock solution with Coating buffer (solution 1) before use. ▪ The concentration is dependent on the plate material used. ▪ Recommended concentration: 2 µg/ml.	Store 2 days at +2 to +8°C.
5	Blocking solution	1% Blocking reagent (w/v) in Basic buffer (solution 2), 0.1% sodium azide (w/v).	Store 4 weeks at +2 to +8°C.
6	Wash buffer	Basic buffer (solution 2) with 0.1% Tween 20 (v/v).	Store 1 week at +2 to +8°C.
7	Sample solution	Dilute samples in solution 3.	Store 2 days at +2 to +8°C.
8a	Anti-Digoxigenin-POD, Fab fragments	▪ Reconstitute according to Instructions for Use. ▪ Working solution in buffer III. ▪ Recommended concentration range: 100 to 250 mU/ml.	Store 2 weeks at +2 to +8°C.
8b	Anti-Digoxigenin-AP, Fab fragments	▪ Reconstitute according to Instructions for Use. ▪ Working solution in solution 3. ▪ Recommended concentration range: 100 to 250 mU/ml.	
9a	Substrate buffer for Anti-Digoxigenin-POD, Fab fragments	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.
9b	Substrate buffer for Anti-Digoxigenin-AP, Fab fragments	1 M diethanolamine, 0.5 mM $MgCl_2$, pH 9.8.	
10a	Substrate solution for Anti-Digoxigenin-POD, Fab fragments	Dissolve 100 mg ABTS Substrate in 100 ml substrate buffer (solution 9a).	Store 3 months at +2 to +8°C. ⚠ ***Store protected from light.***
10b	Substrate solution for Anti-Digoxigenin-AP, Fab fragments	Dissolve 371 mg 4-nitrophenyl phosphate × 6 H_2O in 100 ml substrate buffer (solution 9b).	Store 3 days at +2 to +8°C.

2.2. Protocols

Sample Materials

Use the following sample materials and concentrations when setting up the ELISA.

- Nucleic acids in which digoxigenin-11-dUTP is incorporated by random primed labeling or by nick translation are used at a concentration of 5 to 50 ng/ml, with at least 5 different concentrations.
- Proteins labeled with Digoxigenin-3-0-methylcarbonyl-ε-aminocaproic acid N-hydroxysuccinimide ester* are used at a concentration of 0.5 to 10 ng/ml, with at least 5 different concentrations.
- Proteins labeled with digoxigenin-3-0-succinyl-ε-aminocaproic acid hydrazide hydrochloride, after oxidation of their sugar residues are used at a concentration of 50 to 1,000 ng/ml with at least 5 different concentrations.

If possible, one series of experiments should be performed on one microplate. It is recommended to perform 3-fold determinations for standards and samples.

If available, all suction and washing steps listed below can also be performed with a microplate washer, such as Easy Washer, SLT Labinstruments.

ELISA Assay

1. Pipette 0.25 ml coating solution (solution 4b) 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 by suction or tapping on filter paper.
   - Pipette 0.25 ml blocking solution (solution 5) into the wells and incubate for 15 minutes at +37°C.

3. Remove the contents of the microplate by suction or tapping on filter paper.
   - Pipette 0.2 ml of the sample solution (solution 7) into the wells and incubate for 1 hour at +37°C.

4. Remove the contents of the microplate carefully by suction or tapping on filter paper.
   - Wash wells at least 3 times with wash buffer (solution 6), then carefully remove the remaining wash buffer.

5. Pipette 0.2 ml Anti-digoxigenin-POD, Fab fragments (solution 8a) or Anti-digoxigenin-AP, Fab fragments (solution 8b) into the wells.
   - Cover plate tightly and incubate for 1 hour at +37°C.

6. Remove the contents of the microplate carefully by suction or tapping on filter paper.
   - Wash wells at least 3 times with wash buffer (solution 6), then carefully remove remaining wash buffer.

7. Pipette 0.2 ml substrate solution (solutions 10a or 10b) into the wells and incubate at +15 to +25°C until color development is sufficient for photometric evaluation.

8. Read absorbance against substrate solutions 10a and 10b at 405 nm.

2.3. Parameters

Specificity

The antibody reacts with free and bound digoxigenin.

Working Concentration

Preparation of Antibody Dilution

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

Application	Dilution	Concentration [µg/ml]	Sufficient for
In situ hybridization	1:250 – 1:500	0.4 – 0.2	5,000 – 10,000 *in situ* hybridizations
Immunoblotting (western blot)	1:50 – 1:200	2 – 0.5	4 – 20 blots with 10 ml incubation volume
Immunohistochemistry	1:50 – 1:200	2 – 0.5	1,000 – 4,000 sections
ELISA	1:25 – 1:50	4 – 2	125 – 250 tests

3. Results

The measured absorbance is plotted on the ordinate against the standard concentrations on the abscissa on semi-logarithmic graph paper.

4. Additional Information on this Product

4.1. Test Principle

Antibody Production

To obtain monoclonal antibodies, Balb/c mice were immunized with edestin-bound digoxigenin and the spleen cells were fused with myeloma cells of the cell line P3X63Ag8.653. The antibody was purified by gel filtration, diluted in 10 mM potassium phosphate buffer, 75 mM NaCl, 5% raffinose (w/v), 0.01% 2-methylisothiazolone (MIT) (v/v), pH 7.8, and lyophilized. The antibody belongs to the IgG1, $_{\kappa}$ subclass.

Sandwich-ELISA

The following steps describe the application of the antibody in an ELISA. The grade of digoxigenin-labeling can be determined quantitatively.

1. Coat microplate wells with anti-digoxigenin.
2. Block nonspecific adsorption sites with blocking solution.
3. Incubate the appropriate diluted digoxigenin-labeled compound in the coated microplate.
4. Remove the sample from the plate.
5. After washing, add an enzyme-conjugated anti-digoxigenin (with peroxidase (POD), Fab fragments or with alkaline phosphatase (AP), Fab fragments, which reacts with the digoxigenin-labeled compound bound to the coated monoclonal antibody.
6. Visualize the complexed enzyme using a soluble substrate, such as ABTS Substrate perborate system for peroxidase (POD), or 4-nitrophenyl phosphate for alkaline phosphatase (AP).

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

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

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Anti-Digoxigenin-POD, Fab fragments	150 U	11 207 733 910
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
Digoxigenin-3-O-methylcarbonyl-ε-aminocaproic acid-N-hydroxysuccinimide ester	5 mg	11 333 054 001
ABTS	2 g	10 102 946 001
Blocking Reagent	50 g	11 096 176 001
Tween 20	50 ml, 5 x 10 ml	11 332 465 001
Tris hydrochloride	500 g	10 812 846 001

5.4. Trademarks

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

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany