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

Anti-Digoxigenin-POD (poly), Fab fragments
from sheep

Version: 07
Content Version: December 2020

For the detection of digoxigenin-labeled compounds.
Lyophilizate

Cat. No. 11 633 716 001 50 U

Store the conjugate at +2 to +8°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Anti-Digoxigenin-POD (poly), Fab fragments	1 vial, 50 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.

Vial / Bottle	Label	Storage
1	Anti-Digoxigenin-POD (poly), Fab fragments	Store at +2 to +8°C.

Reconstitution

Dissolve the lyophilizate in 1 ml of double-distilled water; this results in a concentration of 50 U/ml.
The reconstituted stock solution is stable at +2 to +8°C for 6 months.
⚠ ***Do not freeze.***
⚠ ***Do not add sodium azide.***

1.3. Additional Equipment and Reagent required

For ELISA

- ABTS Substrate*
- BM Blue POD Substrate, soluble*
- TMB
- BM Chemiluminescence ELISA Substrate (POD)*

For Immunoblotting

- DAB*
- CN
- BM Chemiluminescence Western Blotting Substrate (POD)*

1.4. Application

The conjugate can be used for the detection of digoxigenin-labeled compounds, such as:
- Digoxigenin-labeled nucleic acids
- Digoxigenin-labeled proteins

Applications include:
- ELISA
- Immunoblotting
- Southern blots, dot blots, western blots

⚠ ***The Anti-digoxigenin-POD(p) conjugate has not been evaluated in immunohistochemistry.***

2. How to Use this Product

2.1. Before you Begin

General Considerations

Substrates for the Detection of POD in ELISA and Immunoblotting Applications

Application	Chromogenic Substrates	Chemiluminescent Substrates
ELISA	**ABTS* (2,2 Azino-di-(3-ethylbenzthiazoline-sulfonate[6])** ▪ 100 mg of 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 reaction product is green and soluble in water; measure at 405 nm. **BM Blue POD Substrate, soluble*** **TMB (3,3′,5,5′ Tetramethylbenzidine) in buffer solution, ready-to-use** ▪ The reaction product is blue (yellow when reaction stopped), and soluble in water; measure at 450 nm.	**BM Chemiluminescence ELISA Substrate (POD)*** ▪ Use according to the Instructions for Use.
Immunoblotting	**DAB Substrate* (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 reaction product is a brown, water-insoluble precipitate. It is also insoluble 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 a small volume of methanol. ▪ The reaction product is a bluish-black, water-insoluble precipitate; it is however, soluble in ethanol.	**BM Chemiluminescence Western Blotting Substrate (POD)*** ▪ Use according to the Instructions for Use.

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

Immunoblotting

Dilute the reconstituted stock solution (50 U/ml) of the Anti-Digoxigenin-POD(p) in 100 mM Tris-HCl; 150 mM NaCl; pH 7.5. If necessary, 1% blocking reagent (w/v), 1 to 5% heat inactivated fetal calf serum (FCS) (v/v), or normal sheep serum can be added to the conjugate dilution buffer for reduction of nonspecific binding.

2.2. Parameters

Working Concentration

Working concentration of conjugate will depend on the application and substrate. The following concentrations should be taken as a guideline:

Application	Dilution	Concentration [mU/ml]	Sufficient for:
ELISA			
Detection of digoxigenin-labeled nucleic acids	1:5,000 – 1:25,000	10 – 2	25,000 – 125,000 tests
Detection of digoxigenin-labeled proteins	1:1,000 – 1:5,000	50 – 10	5,000 – 25,000 tests
Immunoblotting			
Detection of digoxigenin-labeled nucleic acids on membranes (Southern blots, dot blots), and of digoxigenin-labeled proteins on membranes (western blots, dot blots).	1:500 – 1:1,000	100 – 50	4,000 – 8,000 cm^2 of membrane

3. Results

The advantage of anti-digoxigenin-POD(poly) compared with the unpolymerized conjugate is that it usually gives considerably higher signal-to-noise values (Figure 1). It is especially useful when high sensitivity is demanded.

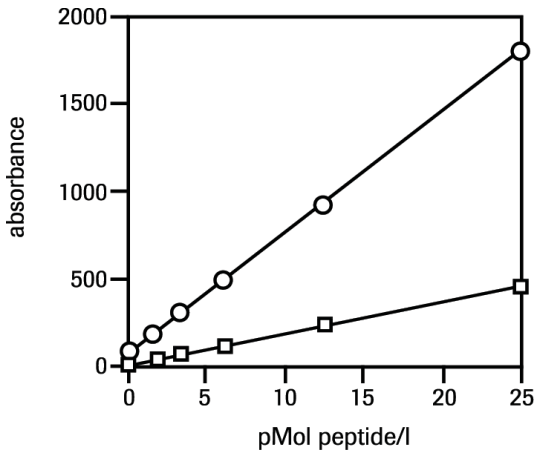

Fig. 1: Comparison of the Anti-Digoxigenin-POD (poly)-conjugate (○, 100 mU/ml) versus the corresponding POD (mono)-conjugate (□, 200 mU/ml).

4. Additional Information on this Product

4.1. Test Principle

The reagent is an anti-digoxigenin antibody from sheep, Fab fragments, conjugated with polymerized horseradish peroxidase [POD(p)]. After immunization with digoxigenin, the sheep IgG was purified by ion-exchange chromatography. The Fab fragments obtained by papain digestion were isolated by immunosorption, conjugated with POD(p), and stabilized in a 50 mM HEPES buffer, 0.4% bovine serum albumin (BSA) (w/v), 0.1% methylisothiazolone (MIT) (w/v); pH 7.4, and lyophilized.

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		
ABTS	2 g	10 102 946 001
BM Chemiluminescence ELISA Substrate (POD)	250 ml, 2,500 wells or 1,000 tubes	11 582 950 001
BM Chemiluminescence Western Blotting Substrate (POD)	1 set, 1,000 cm^2 membrane (trays), 6,250 cm^2 membrane (transparent plastic bags)	11 500 708 001
	1 set, 4,000 cm^2 membrane (trays), 25,000 cm^2 membrane (transparent plastic bags)	11 500 694 001
DAB Substrate	1 pack	11 718 096 001
BM Blue POD Substrate, soluble	100 ml	11 484 281 001

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.

1220.11632159001 ©

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim
Germany