For general laboratory use. Not for use in diagnostic procedures. FOR *IN VITRO* USE ONLY.

Anti-Digoxigenin-POD (poly), Fab fragments

For Detection of Digoxigenin-labeled Compounds
Lyophilisate

Cat. No. 11 633 716 001
50 U

Version February 2006
Store at +2 to +8°C

Product Description

Preparation

The reagent is an anti-digoxigenin antibody from sheep, Fab fragments, conjugated with polymerized horse-radish 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% Methylisothialzolone (MIT) (w/v); pH 7.4 and lyophilized.
Dissolving the lyophilisate in 1 ml redist. water results in a concentration of 50 U/ml.

Advantage

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

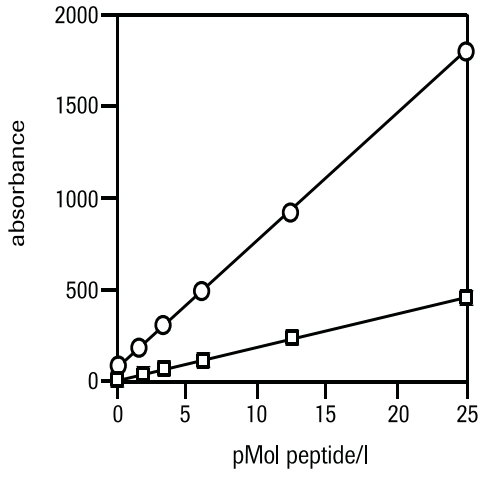

Fig. 1: Comparison of the anti-digoxigenin-POD(poly)-conjugate (○ 100 mU/ml) versus the corresponding POD(mono)-conjugate (□ 200 mU/ml).

Storage and Stability

The conjugate is stable at +2 to +8°C. The reconstituted stock solution is stable at+2 to +8°C for 6 months; do not freeze. Do not add sodium azide!

Recommended Concentrations

1. ELISA

Detection of digoxigenin-labeled nucleic acids:

1:5000 - 1:25 000 = 10 mU/ml - 2 mU/ml, sufficient for 25 000 - 125 000 tests.

Detection of digoxigenin-labeled proteins:

1:1000 - 1:5000 = 50 mU/ml - 10 mU/ml, sufficient for 5000 - 25 000 tests.

2. Immunoblotting

Detection of digoxigenin-labeled nucleic acids (Southern blots, dot blots) and of digoxigenin-labeled proteins (Western blots, dot blots) on membranes.

1:500 - 1:1000 = 100 mU/ml - 50 mU/ml, sufficient for 4000-8000 cm^2 of membrane.

Note: It is recommended to 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), 1-5% heat inactivated fetal calf serum (FCS) (v/v) or sheep normal serum can be added to the conjugate dilution buffer for reduction of non-specific binding.

The anti-digoxigenin-POD(p) conjugate has not been evaluated in immunohistochemistry.

Substrates for the Detection of POD in ELISA and Immunoblotting Applications

1. ELISA

Chromogenic Substrates

ABTS*, [2,2 Azino-di-(3-ethylbenzthiazoline-sufonate(6))]
100 mg of ABTS in 3.25 mM sodium perborate; 39.8 mM citric acid; 60 mM disodium hydrogen phosphate; pH 4.4 - 4.5.
The reaction product is green and soluble in water; measure at 405 nm.

(for the Cat. Nos. please refer to our homepage or catalogue)

BM blue POD substrate, soluble,
Cat. No. 11 484 281 001

TMB (3,3´,5,5´ Tetramethylbenzidine) in buffer solution, ready-to-use.
The reaction product is blue (after stop yellow) and soluble in water; measure at 450 nm.

Chemiluminescent Substrate

BM Chemiluminescence ELISA Substrate (POD)*,
Cat. No. 11 582 950 001

Use according to the pack insert.

2. Immunoblotting

Chromogenic Substrates	DAB (Diaminobenzidine (3,4,3',4'-tetraaminobiphenyl)) Cat. No. 11 718 096 001 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.
	BM blue POD substrate, precipitating Cat. No. 11 442 066 001 TMB (3,3´,5,5´ Tetramethylbenzidine) in buffer solution, ready-to-use. The reaction product is a dark blue precipitate which is insoluble in water.
	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 methanol. The reaction product is a bluish-black, water-insoluble precipitate, which is however, soluble in ethanol.
Chemiluminescent Substrate	BM Chemiluminescence Western Blotting Substrate (POD), Cat. No. 11 500 708 001 Use according to the pack insert.

* *available from Roche Applied Science*
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Ordering Information

Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page www.roche-applied-science.com.

Notice for the Purchaser	The labeling of nucleic acids with DIG is covered by EP patents 0 324 474 and 0 371 262 as well as the following US patents 5.344.757, 5.354.657 and 5.702.888 owned by Roche Diagnostics GmbH.

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