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

Peroxidase Labeling Kit
from horseradish

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Kit for the labeling of primary amino groups of biomolecules with activated peroxidase (POD).

Cat. No. 11 829 696 001 1 kit
5 labeling reactions

Store the kit at +2 to +8°C.

1. General Information

1.1. Contents

Vial / bottle	Cap	Label	Function / description	Content
1	white	Peroxidase Labeling Kit, POD activated	Lyophilized, stabilized	1 vial, 8 mg
2	blue	Peroxidase Labeling Kit, Sodium carbonate buffer	1 M Sodium carbonate/ hydrogencarbonate buffer, pH 9.4	1 bottle, 100 ml
3	white	Peroxidase Labeling Kit, Natrium borhydrid	Natrium borhydrid	1 bottle, 6 tablets
4	violet	Peroxidase Labeling Kit, Triethanolamine solution	2 M Triethanolamine buffer, pH 8.0	1 vial, 0.5 ml
5	yellow	Peroxidase Labeling Kit, Glycine solution	Lyophilized *1 M solution after addition of 0.5 ml double-distilled water.*	1 vial
6	green	Peroxidase Labeling Kit, Dialysis buffer, 20x conc.	Dialysis buffer, pH 6.5	2 bottles, 100 ml each
7	black	Peroxidase Labeling Kit, Stabilizing reagent	Stabilizing agent with BSA and Kathon CG.	1 bottle, 3 ml

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Cap	Label	Storage
1	white	POD activated	Store at +2 to +8°C.
2	blue	Sodium carbonate buffer	
3	white	Natrium borhydrid	
4	violet	Triethanolamine solution	
5	yellow	Glycine solution	
6	green	Dialysis buffer, 20x conc.	
7	black	Stabilizing reagent	

1.3. Additional Equipment and Reagent required

For preparation of solutions

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

- Double-distilled water

For conjugation

- Kathon, CG
- Sephadex G25 or PD columns
- Water bath
- Dialysis tube (boiled water treated)
- HPLC TSK-3000 columns
- Chromatography equipment

1.4. Application

The Peroxidase Labeling Kit can be used for:

- Labeling water-soluble substances with reactive and accessible primary amino groups, for example, peptides or proteins with peroxidase for use in analytical methods.
- It is particularly suitable for the coupling of antibodies with peroxidase, as the resulting conjugate is used in immunochemical detection systems, such as ELISA, immunohistochemistry, and immunoblotting procedures.

The quantity of kit reagents is sufficient to conjugate approximately 6 mg IgG. Aliquot the total quantity to be conjugated into 5 portions; each portion will provide 0.5 ml to 1 ml of conjugate from approximately 1.2 mg IgG, a quantity that can be diluted 1:4,000 to 1:10,000 for ELISA and blotting applications.

2. How to Use this Product

2.1. Before you Begin

General Considerations

Purification/fractionation of the conjugate

- During the reaction, more than 75% of the IgG is conjugated and only 20% of the immunoglobulin remains uncoupled (Figure 3).The conjugate does not need to be purified for normal immunoassay procedures. Residual amounts of free peroxidase after conjugation do normally not interfere with such procedures.
- When the conjugate is to be used for special applications, such as highly sensitive ELISA procedures or measurements in complex matrices, it can be further purified subsequent to dialysis and prior to stabilization with Solution 7 (Stabilizing reagent), by gel permeation chromatography, and the fractions tested for their suitability for the planned application.

Rebuffering of antibody and conjugate

See section, **Protocols** for additional information about Steps 4 and 5.
The antibody can be rebuffered into Solution 2 either using Sephadex G25 or PD-columns or other suitable material. The conjugate can then be rebuffered subsequent to Step 4 using column chromatography, instead of the described dialysis (Step 5).

Reaction ratio IgG to POD

See section, **Protocols** for additional information about Step 1.
The conjugation of IgG and fab-fragments is optimized for the Step 1 ratio. Other stoichiometric proportions can be considered for special applications, but the protein concentrations indicated in the test procedure should be held constant and a molecular sieve fractionation should be carried out to separate residual IgG or POD.

Immunoglobulin

The procedure is optimized for coupling immunoglobulin from rabbit; it can also be conjugated with IgG from sheep and goat. When Fab- or $F(ab')_2$-fragments of these species or immunoglobulin G from mouse are to be used, a reaction time of 3 hours at +15 to +25°C should be used.

Influence of NaCl and potassium phosphate concentration

NaCl concentrations of 50 to 400 mM and simultaneous phosphate concentrations of 10 to 20 mM have a marginal effect on the reaction.
- For potassium phosphate, use a concentration in the range of 30 to 100 mM.
- The NaCl concentration should not exceed 150 mM.

HPLC chromatography

For high reproducibility, reactions should be carried out under HPLC TSK-3000 control. Figures 1 to 3 show the TSK-3000 profiles of the starting material, immunoglobulin G from rabbit (Figure 1), POD (Figure 2), and the final product (Figure 3). The IgG is completely bound in the conjugate, corresponding to the TSK-3000 separation profile of Figure 3.

2. How to Use this Product

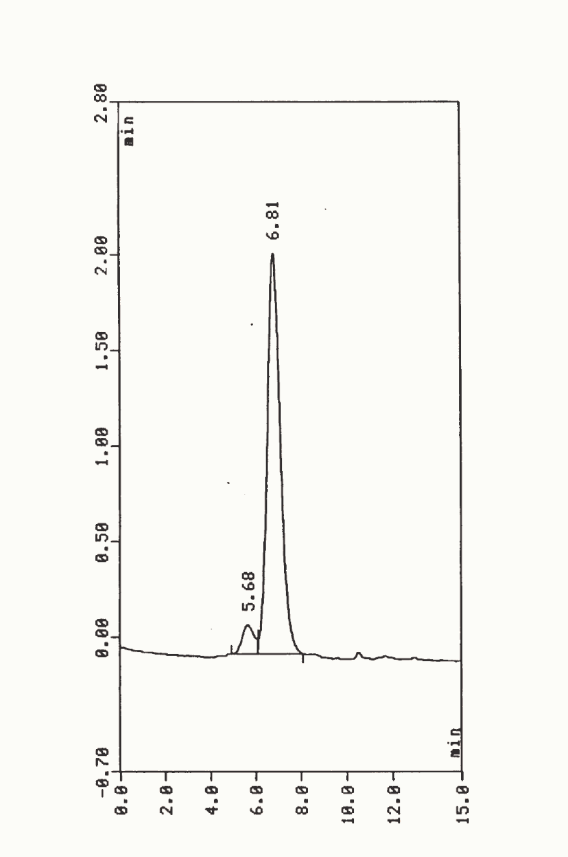

Fig. 1: HPLC, TSK-3000; IgG from rabbit.

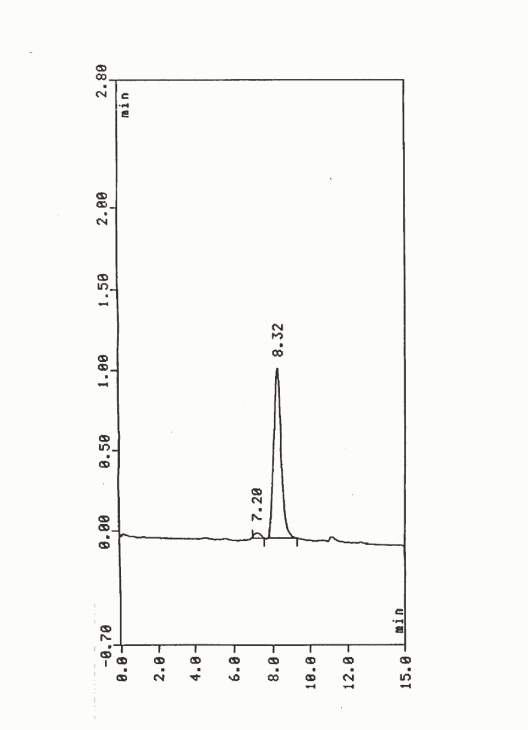

Fig. 2: HPLC, TSK-3000; Peroxidase (POD).

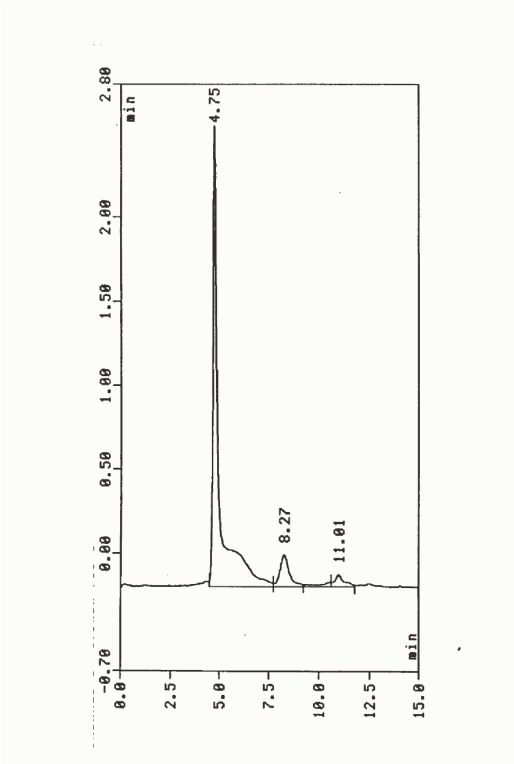

Fig. 3: HPLC, TSK-3000; Conjugate of IgG and POD.

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

To avoid confusion, mark each solution with the appropriate number. The volumes listed are sufficient to label a portion of 1.2 mg IgG.

Solution No.	Solution	Preparation/Composition [+15 to +25°C]	Storage and Stability
1	Peroxidase, activated	Reconstitute the lyophilizate (Bottle 1) in 0.5 ml double-distilled water. *Peroxidase concentration = 16 mg/ml.*	Store 3 months at +2 to +8°C, or in aliquots shock frozen at −60°C or below and then stored at −15 to −25°C. ***A 10 to 20% loss of activity is observed.***
2	100 mM Sodium carbonate/-hydrogencarbonate buffer, pH 9.8	▪ Equilibrate Bottle 2 (Sodium carbonate buffer) to +15 to +25°C. Make sure all buffer components are dissolved. ▪ Add 10 ml of Bottle 2 to 90 ml double-distilled water; mix well.	Store 1 week at +2 to +8°C or in aliquots for 6 months at −15 to −25°C.
3	200 mM Natrium borhydrid	Add 1 tablet of Bottle 3 to 130 ml cold, double-distilled water; mix well. ***Always wear gloves when working with Natrium borhydrid.***	***Prepare immediately before use. Keep cold on ice.***
4	2 M Triethanolamine, pH 8.0	▪ Bottle 4 is ready-to-use. ▪ Equilibrate Bottle 4 to +15 to +25°C. Make sure all buffer components are dissolved.	Store at +2 to +8°C until the expiry date on the kit.
5	1 M Glycine, pH 7.0	Reconstitute the lyophilizate (Bottle 5) in 0.5 ml double-distilled water. *Glycine concentration = 1 M.*	Store 1 week at +2 to +8°C or in aliquots for 6 months at −15 to −25°C.
6	Dialysis buffer, 1x conc.	▪ Equilibrate Bottle 6 to +15 to +25°C. Make sure all buffer components are dissolved. ▪ Add 30 ml Bottle 6 to 570 ml double-distilled water; mix well.	
7	Stabilizing reagent	▪ Bottle 7 is ready-to-use. ▪ Equilibrate Bottle 7 to +15 to +25°C.	Store at +2 to +8°C until the expiry date on the kit.
8	Antibody solution	▪ 0.3 ml is required for each labeling reaction. ▪ The IgG concentration of the solution to be used is c = 4 mg/ml (3.8 to 4.2 mg/ml). ***This value is critical for the coupling and should be checked photometrically for every test and adjusted if necessary: A_{280nm}, 1 cm, 1 mg/ml = 1.40.*** *Do not use preservatives, such as sodium azide, and stabilizers, such as albumin or detergents.* **Immunoglobulin, salt-free, lyophilized**: ▪ Weigh 1.6 mg into a suitable vessel and dissolve in 0.4 ml Solution 2. ▪ Check the concentration and pH and correct if necessary. **Immunoglobulin in buffer**: ▪ PBS buffer without additional proteins or preservatives: adjust the pH to 9.8 with 1 M Sodium carbonate buffer (Bottle 2) and if necessary, dilute with Solution 2 to obtain an IgG concentration of 4 mg/ml. ▪ Buffer with organic salts: Dialyze immunoglobulin into Solution 2 and adjust the concentration to 4 mg/ml with Solution 2.	***Prepare immediately before use. Keep cold on ice.***

2.2. Protocols

Conjugation

The Conjugation protocol has been specially developed for the coupling of peroxidase to immunoglobulin G (IgG). It can be equally successfully used for IgG Fab- and F(ab')2-fragments from rabbit, mouse, sheep and goat, see section, **General Considerations**. If other proteins are to be conjugated, start with this procedure and check the results with gel chromatography on HPLC, TSK-3000. If necessary, the procedure can then be adapted to individual requirements by altering the stoichiometry and the concentration of reactants used for incubation. The test procedure describes the conjugation using 1/5 of the total quantity of kit reagents, sufficient to label 1.2 mg of IgG.

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

1. Pipette exactly 0.3 ml Antibody solution into a suitable 1 to 2 ml vessel, add 0.1 ml Solution 1 (activated Peroxidase), and mix well.
   – Reaction ratio: 1 M IgG:5 M POD.
   Molecular weight of POD is 44,000 Da.

2. Incubate for 2 hours at +15 to +25°C in a water bath or for 16 hours overnight at +2 to +8°C.

3. Stop the reaction by adding 40 µl Solution 4 (Triethanolamine solution) to the incubation solution and mix.
   – Pipette 50 µl Solution 3 (Natrium borhydrid solution) to the mixture, mix again, and incubate for 30 minutes at +2 to +8°C.
   – Add another 25 µl of Solution 4 (Triethanolamine solution) and incubate again for 2 hours at +2 to +8°C.

4. Stabilize the conjugate by pipetting 10 µl Solution 5 (1 M Glycine solution) into the incubation mixture; mix well.

5. Transfer conjugate to storage buffer by placing the incubation solution in a dialysis tube (boiled-water treated) and dialyze extensively, for example, overnight with 4 changes of 200 ml Solution 6 (1x Dialysis buffer).

6. Stabilize the product for storage by placing the conjugate from the dialysis tube into a suitable vessel, determine the volume, and add the same volume of Solution 7 (Stabilizing reagent) to the conjugate; mix gently.

7. Store the conjugate for at least 2 months at +2 to +8°C.
   – For long-term storage, aliquot and shock freeze in liquid nitrogen and store at −60°C or below.

⚠ ***Never add sodium azide as a preservative to the Natrium borhydrid solution, as it inhibits the peroxidase activity.***

2.3. Parameters

Inhibition

Never add sodium azide as a preservative as it inhibits peroxidase activity.

pH Stability

The pH should not be allowed to fall below 9.8 during the conjugation reaction, see section, **Protocols**. To ensure reproducible results, keep pH constant to ensure proper coupling.
⚠ ***The maximum usable pH is 10.8.***

Purity

Purity number ($A_{405\ nm}/A_{275\ nm}$): 3.0 to 3.5
Isoenzyme distribution: >90% homogeneous isoenzyme C.

Specific Activity

≥550 U/mg protein at +25°C with ABTS Substrate, H_2O_2, pH 5.0.

Temperature Stability

Reaction temperature and duration

The procedure is developed for a reaction temperature of +25°C, but is reaction time tolerant.
- At +15 to +25°C, the reaction time should be 2 hours, but this can be extended to 3 hours without influencing the results.
- Reactions can also be carried out at +2 to +8°C for 18 to 24 hours.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section **Contact and Support**.

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. Trademarks

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

4.4. License Disclaimer

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

4.5. Regulatory Disclaimer

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

4.6. Safety Data Sheet

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

4.7. 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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