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

Peroxidase Labeling Kit

Kit for the labeling of primary amino groups of biomolecules with activated peroxidase (POD) from horse radish

Cat. No. 11 829 696 001

Kit for 5 labeling reactions

Version 07

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Store at +2 to +8°C

Number of Reactions

8 mg activated peroxidase for labeling of approx. 6 mg protein in 5 reactions

Kit contents

Bottle	Kit contents	Amount	Color code
1	Peroxidase (activated) (POD), stabilized lyophilizate	8 mg	white cap
2	1 M Sodium Carbonate/ hydrogencarbonate-buffer, pH 9.4	100 ml	blue cap
3	Sodium Borohydride	6 tablets	white cap
4	2 M Triethanolamine Buffer, pH 8.0	0.5 ml	violet cap
5	1 M Glycine Solution, pH 7.0	0.5 ml after recon-stitution	yellow cap
6	Dialysis Buffer, 20 × conc. pH 6.5	2 × 100 ml	green cap
7	Stabilizing Agent with BSA and Kathon CG	3 ml	black cap

Advantages of the kit

- **fast** (the whole labeling procedure can be performed in one day)
- **easy** (only 5 simple working steps)
- **complete** (all necessary buffers and reagents are supplied with the kit)

1. Application

The kit is designed for labeling water-soluble biomolecules with reactive and accessible primary amino groups (e. g. peptides or proteins) with peroxidase for use in analytical methods.

It is particulary suitable for the coupling of antibodies with peroxidase, as the resulting conjugate is optimal for use in immunochemical detection systems like ELISA, immunohistochemistry and immunoblotting.

2. Kit characteristics

2.1 Capacity

The quantity of kit reagents is sufficient to conjugate approx. 6 mg immunoglobulin G (IgG). We recommend to aliquot the total quantity to be conjugated into 5 portions. Each portion will then provide 0.5 – 1 ml of conjugate from approx. 1.2 mg IgG, a quantity that can be diluted 1 : 4 000 – 1 :10 000 for ELISA and blotting applications

2.2 Specification of the peroxidase

Specific activity: ≥ 550 U/mg protein at +25°C and pH 5.0 with ABTS* and H_2O_2 as substrates. Purity number: (A_{405nm}/A_{275nm}): 3.0 – 3.5. Isoenzyme distribution: > 90% homogeneous isoenzyme C.

2.3 Stability

The kit is stable until the expiration date printed on the label if stored at +2 to +8° C. For stability of working solutions see chapter 3.1.2.

3. Labeling procedure

The following procedure has been specially developed for the coupling of peroxidase to immunoglobulin G (IgG). It can however be as well used for IgG, Fab and $F(ab')_2$ fragments from rabbit, mouse, sheep and goat (see chapter 4. notes). The test procedure describes the conjugation using 1/5 of the total quantity of the kit reagents, sufficient to label 1.2 mg of IgG.

If other proteins are to be conjugated, we would recommend beginning with this procedure and checking the results with gel chromatography on HPLC with 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.

3.1 Preparation of solutions

All neccessary reagents for performing the coupling are contained in the kit. Redist. water should always be used for reconstitution and dilution purposes.

Additional required materials: dialysis tube (boiled water treated)

3.1.1 Preparation of working solutions

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

Solution 1: Peroxidase (activated)

Reconstitute the lyophilizate of bottle 1 in 0.5 ml redist. water (peroxidase concentration = 16 mg/ml)

Solution 2: Sodium Carbonate/-hydrogencarbonate Buffer (100 mM, pH 9.8)

Equilibrate bottle 2 to +15 to +25° C. Be sure that all buffer components are dissolved. Add 10 ml of bottle 2 to 90 ml redist. water; mix well.

Solution 3: Sodium Borohydride Solution (200 mM)

We recommend to wear gloves when working with sodium borohydride. Prepare the solution immediately prior to use and keep cold on ice. Add 1 tablet of bottle 3 to 130 ml cold, redist. water; mix well.

Solution 4: Triethanolamine Buffer (2 M, pH 8.0)

Bottle 4, ready-to-use. Equilibrate the bottle to +15 to +25° C. Be sure that all buffer components are dissolved.

Solution 5: Glycine Solution (1 M, pH 7.0)

Reconstitute the lyophilizate of bottle 5 in 0.5 ml redist. water (glycine concentration = 1 M)

Solution 6: Dialysis Buffer (1 × conc.)

Equilibrate bottle 6 to +15 to +25° C. Be sure that all buffer components are dissolved. Add 30 ml of bottle 6 to 570 ml redist. water; mix well.

Solution 7: Stabilizing Agent

Bottle 7 is ready-to-use. Equilibrate the bottle to +15 to +25° C.

3.1.2 Stability of solutions

- Solution 1 is stable for 3 months at +2 to +8° C. The solution can be aliquoted, shock-frozen at −60° C or below and then stored at −15 to −25° C; however, a loss o factivity of 10 – 20% may be observed.
- Solutions 2,5 and 6 are stable for 1 week at +2 to +8° C, respectively for 6 months at −15 to −25° C, if stored frozen in aliquots.
- Solution 3 should always be prepared immediately before use.
- Solution 4 and 7 are stable at +2 to +8° C until the expiry date stated on the kit.

3.2 Preparation of the immunoglobulin solution

The IgG concentration of the antibody solution should be approx. 4 mg/ml (3.8 – 4.2 mg/ml). This concentration is critical for the coupling and should hence be checked photometrically before every coupling and adjusted if necessary: 1 mg/ml = 1.40 at $A_{280\ nm}$ and 1 cm path length. 0.3 ml of this solution are required for each labeling reaction.
Note: Do not use preservatives like sodium azide and stabilizers like albumin or detergents.

3.2.1 Lyophilized immunoglobulin, salt-free

Weigh 1.6 mg into a suitable vessel and dissolve in 0.4 ml solution 2. Check the concentration and pH and adjust if necessary.

3.2.2 Immunoglobulin in buffer

If the immunoglobulin is dissolved in phosphate buffered saline (PBS) without additional proteins or preservatives: adjust the pH to 9.8 with 1 M sodium carbonate buffer (bottle 2). If necessary dilute with solution 2 to obtain an IgG concentration of 4 mg/ml.

If the immunoglobulin is dissolved in a buffer with organic salts: Dialyse immunoglobulin with solution 2 and adjust the concentration to 4 mg/ml with solution 2.

3.2.3 Stability of IgG solution

The solutions should always be prepared immediately for use.

3.3 Labeling protocol

3.3.1 Conjugation

Pipette exactly 0.3 ml antibody solution into a suitable 1 – 2 ml vial and add 0.1 ml solution 1 (peroxidase); mix well. Reaction ratio = 1 M IgG : 5 M POD. Incubate for 2 h at 25°C in a water bath or for 16 h overnight at +2 to +8° C.

3.3.2 Stopping the reaction

Add 40 µl solution 4 (triethanolamine) to the incubation solution, mix, pipette 50 µl solution 3 (sodium borohydride) to the mixture, mix again and incubate for 30 min at 2-8°C. Add a further 25 µl of solution 4 and incubate again for 2 h at +2 to +8° C.

3.3.3 Stabilizing the conjugate

Pipette 10 µl of solution 5 (glycine solution) into the incubation solution and mix well.

3.3.4 Transfer of conjugate to storage buffer

Place the incubation solution in a dialysis tube and allow to dialyse extensively (e.g. overnight) with 3 changes of 200 ml solution 6 (1 × dialysis buffer).

3.3.5 Stabilizing the product for storage

Place the conjugate from the dialysis tube in a suitable vial, determine the volume and add the same volume of solution 7 to the conjugate; mix gently. The conjugate is stable for at least 2 months at +2 to +8° C. For longer storage the conjugate can be aliquoted, shock-frozen in liquid nitrogen and then stored at −60° C or below.

4. Notes

4.1 Sodium azide

Sodium azide should never be added as a preservative, because it inhibits the peroxidase activity.

4.2 Purification and fractionation of the conjugate

During the reaction more than 75% of the IgG is conjugated and only 20% of the immunoglobulin remains uncoupled (see Fig.3). The conjugate needs therefore not be purified for normal immunoassay procedures. Residual amounts of free peroxidase after conjugation do normally not interfere with such procedures.

If the conugate is to be used for special applications like highly sensitive ELISA procedures or measurements in problematic matrices, it can be purified further subsequent to dialysis and prior to stabilization with solution 7 by gel permeation chromatography (e.g. on Sephacryl S300 from Pharmacia). The fractions can then be tested for their suitability for the planned application.

4.3 Re-buffering of antibody and conjugate

The antibody can be re-buffered into solution 2 either using Sephadex G25 or PD-columns (Pharmacia) or other suitable material. The conjugate can be re-buffered subsequently to step 3.3.3 by using column chromatography instead of the described dialysis (step 3.3.4)

4.4 Reaction ratio IgG to POD

The conjugation of IgG and Fab-fragments is optimized for the above reaction ratio. Other stoichiometric proportions can be considered for special applications, but it must be stressed that the protein concentration indicated in the test procedure should be held constant and that a molecular sieve fractionation should be carried out in order to separate out residual amounts of IgG or POD that may be present.

4.5 Immunoglobulin

The procedure is optimized for the coupling of immunoglobulin from rabbit; IgG from sheep and goat can likewise be conjugated. If Fab or $F(ab')_2$ fragments of these species or immunoglobulin G from mouse are used, a reaction time of 3 h at +25° C is recommended.

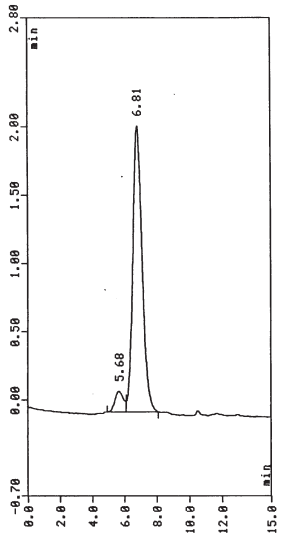

Fig. 1

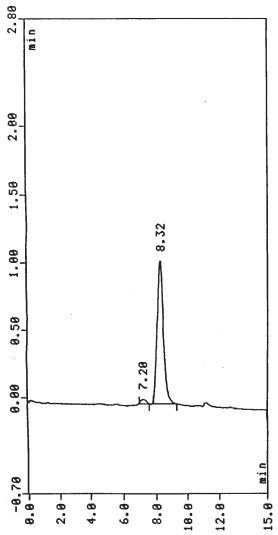

Fig. 2

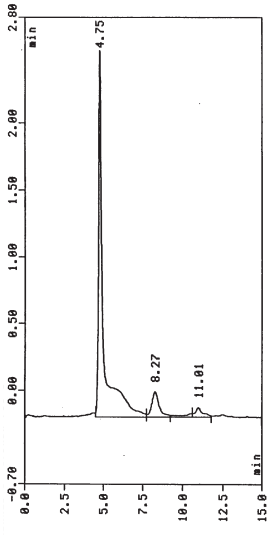

Fig. 3

4.6 Reaction temperature and reaction time

The procedure is developed for a reaction temperature of +25° C. At +25° C the reaction time should be 2 h but it can be extended to 3 h without influencing the results. Alternatively, the reaction can be carried out at +2 to +8° C with a reaction time of 18 h up to 24 h.

4.7 Influence of pH

During the conjugation reaction (step 3.3.1) the pH should never drop below 9.8 and must be kept constant to ensure a proper coupling. The maximally allowable pH is 10.8.

4.8 Influence of NaCl and potassium phosphate concentration

NaCl concentrations of 50 – 400 mM and simultaneous phosphate concentrations of 10 – 20 mM have only a marginal effect on the reaction. If the potassium phosphate concentration is in the range of 30 – 100 mM, the NaCl concentration should not exceed 150 mM.

4.9 HPLC chromatography

If high reproducibility is required, the reaction should be carried out under HPLC-TSK 3000 control. Figures 1 – 3 show the TSK 3000 profiles of the starting material (Fig. 1: immunoglobulin G from rabbit), of POD (Fig. 2) and the final product (Fig. 3). During reaction, the IgG is completely bound in the conjugate, as can be seen in the TSK 3000 separation profile of Fig 3.

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