

Conjugation of antibodies by use of glutaraldehyde

WOLF D. KUHLMANN

*Division of Radiooncology, Deutsches Krebsforschungszentrum, D-69120 Heidelberg
Laboratory Diagnostics & Cell Science, 56112 Lahnstein*

The conjugation of antibodies with HRP by use of glutaraldehyde was introduced by S AVRAMEAS (1969). Glutaraldehyde was earlier employed for tanning in the leather industry (SELIGSBERGER L and SADLIER C 1957) and this aldehyde was then introduced by DD SABATINI et al. (1963) for the fixation of biological specimens in electron microscopy. Fundamental ideas on the reaction of glutaraldehyde with proteins have been published by JH BOWES and CW CATER (1965). Amino acid analyses showed that glutaraldehyde reacts almost exclusively with ϵ -amino groups of proteins.

Conjugation of HRP with antibodies is preferably done in a “two-step” technique (AVRAMEAS, S and TERNYNCK T 1971). In a first step, free ϵ -amino groups of HRP react with one of the aldehyde groups. The configuration of the obtained molecules is such that the second free aldehyde group does not couple with another HRP molecule; thus being available for combination with an amino group of antibody added in the second step. *

Procedure of conjugation of HRP with antibodies

For all labeling purposes, highly purified peroxidase preparations from horseradish (HRP) with **RZ 3** are employed; Reinheitszahl RZ = $A_{403 \text{ nm}} : A_{275 \text{ nm}}$. These preparations are highly active, approx. 250 units/mg, measured with H_2O_2 and guaiacal.

1. 10 mg peroxidase are dissolved in 0.2 mL of 0.1 mol/L phosphate buffer pH 6.8 and mixed dropwise with 0.2 mL of 2% glutaraldehyde (purified) in the same buffer. The reaction mixture is kept for 18 h at room temperature and is continuously stirred.
2. Unreacted aldehydes are eliminated by gel filtration (e.g. Sephadex G 25 fine, equilibrated in 0.15 mol/L NaCl). The brown colored fraction contains the activated peroxidase and this is (if needed) concentrated to 1 mL; a 10-fold molar excess of activated HRP is used for conjugation of antibodies.
3. 1 mL of activated HRP is mixed with 1 mL antibody (5 mg antibody per mL 0.15 mol/L NaCl) and 0.3 mL of 0.5 mol/L carbonate buffer pH 9.5 is added. The mixture is stirred at 4°C for 24 h.
4. The conjugate is then dialyzed against 0.2 ml/L ethanolamine-HCl buffer pH 7.4 (2 x 24 h) and, finally, against PBS (2 x 24 h) at 4°C; sterile filtration through 0.22 μm filter, storage at 4°C.

Purification of conjugates is recommended because unconjugated antibodies will interfere with labeled antibodies by competing for antigenic sites. Mixtures of conjugated antibodies,

* Cross-linking reagents and other chemicals for conjugation purposes or for enzyme measurements can be toxic. They must be handled with care

unconjugated antibodies and free marker molecules can be readily separated on the basis of molecular size sieving by passage through a suitable column bed of granulated gels. To this aim, columns for example with Sephadex G 200 superfine or Sephacryl S-200 may be employed. Furthermore, purification of peroxidase conjugated antibodies is achieved on the basis of immunological bindings (affinity chromatography; 1st step: insolubilized homologous antigen; 2nd step: insolubilized anti-peroxidase antibodies) or on the basis of lectin bindings. In the latter case, we employ a combination of gel filtration and Concanavalin A binding (KUHLMANN WD 1984). Finally, the catalytic activity of peroxidase is measured in the conjugates.

Conjugation of antibodies with other enzymes (e.g. glucose oxidase) can be done in the same manner as described above.

References for further readings

Seligsberger L and Sadler C (1957)
Sabatini DD et al. (1963)
Bowes JH and Cater CW (1965)
Avrameas S (1969)
Avrameas S and Ternynck T (1971)
Kuhlmann WD (1984)

Full citation of publications is given in chapter *References*

link: <https://www.kuhlmann-biomed.de/wp-content/uploads/2020/12/References.pdf>