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ProductInformation

PEROXIDASE MALEIMIDE ACTIVATED

Product No. **P 1709**

Product Description
Peroxidase maleimide activated is designed for facile conjugation to compounds which contain or have been modified to contain sulfhydryl groups.[1,2]

This reagent is pre-activated to contain 1.0-3.0 moles maleimide/mole peroxidase and may be coupled without further modification to any sulfhydryl containing compound. The maleimide/sulfhydryl interaction is highly specific, efficient and results in a stable thioether linkage. If conjugates are desired with a molecule that does not contain a sulfhydryl, one may be incorporated by a number of methods.

- Compounds containing primary amines may be modified with S-acetylthioglycolic acid N-hydroxysuccinimide ester. (SATA, Product No. A 9043) This reagent will incorporate a protected sulfhydryl which may easily be deacetylated to allow for conjugation.[3]

- Disulfide bonds in the Fc portion of antibodies may be reduced using 2-mercaptoethylamine (Product No. M 6500). This generates two fragments containing free sulfhydryls which are distal from the antigen binding sites.[4]

Recommended Method for Coupling
1. Reconstitute P 1709 to 2-5 mg/ml in 0.15 M NaCl, 0.1 M Sodium phosphate pH 7.0. In order to promote efficient conjugation, the buffer should be deaerated and purged with nitrogen or argon before use. Also, the water used to prepare the buffer should be free of trace heavy metals and other oxidizing agents. The coupling may be performed in an amber vial to protect from light which may catalyze oxidation as well.
2. Dissolve the sulfhydryl compound to be conjugated at 2-5 mg/ml in the same buffer used in step 1. Generally 1-2-moles of peroxidase per mole sulfhydryl compound should be used. The molecular weight of peroxidase is 40,000.
   NOTE: If the conjugating compound was SATA activated, initiate coupling by adding N-Hydroxylamine as per reference 3.
3. Stir gently for 3 hours at room temperature. Block any remaining maleimide groups by adding 1 M 2-Mercaptoethanol (Product No. M 6250) to a final concentration of 0.0015 M. Stir for 15 minutes.
5. Block any remaining sulfhydryls by adding 0.3 M N-Ethylmaleimide (Product No. E 3876) in N,N´-Dimethylformamide (DMF, Product No. D 8654) to a final concentration of 0.003 M.
   Some enzymes may lose significant activity if their native sulfhydryl groups are blocked. In this case the sulfhydryl blocking step should be eliminated.
6. The conjugate should now be exchanged into the appropriate buffer by chromatography or dialysis to place it in the desired medium for use. For long term storage, the conjugate should be stored with 50% glycerol at –20 °C.
7. For analysis purposes, approximately 5-10 µg conjugate/ml will provide a sufficient signal for detection.

This procedure will generally allow efficient coupling to a typical protein. Under some conditions, however, a conjugate with higher sensitivity may be required. In this case, a separate step may be added to remove any uncoupled protein.

References
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2. Kitagawa, T., et al., Chem. Pharm. Bull., **29(4),** 1131 (1981).
3. Duncan, R.J.S., et al., Anal. Biochem., **132,** 68 (1983).
4. Palmer, J.L., et al., J. Biol. Chem., **238(7),** 2393 (1963).