

## OPTIMIZATION OF SODIUM PERIODATE CONJUGATION REACTION OF ANTI-HUMAN GOAT IGG AND HORSERADISH PEROXIDASE.

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Oxidation of peroxidase (HRP) polysaccharide residues with sodium peroxidase provides an efficient way of generating reactive aldehyde groups for subsequent coupling with free side chain amine groups from immunoglobulins via reductive amination. Such methodology was first introduced by Nakane and Kawoi (1974) and still remains between the most used conjugation processes. Lost of conjugate activity and sensibility has been described mainly in diluted conjugate solutions. To solve these problems the Nakane and Kawoi's reaction condition were reviewed. The anti-human goat IgG was purified by saline precipitation followed by ion-exchange perfusion chromatography (IEX) in Poros HQ column. High quality commercial peroxidase was used without further purification. The effect of peroxidase oxidation was determined by reactions of HRP in different periodate conditions. Side reactions due to non-reduced aldehyde groups were determined by changing sodium borohydride concentration. The obtained conjugates were purified by anion exchange chromatography in a Poros HQ column or by sieving exclusion chromatography in Superdex 200 column. The purified conjugates whose presented the best results in a standard ELISA assay were analyzed by circular dichroism.