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Preparation of a protein-LG conjugated to horseradish peroxidase by the periodate method.

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ABSTRACT

SpLG comprises of 4 Ig-binding domains of SpL and 2 IgG Fc-binding SpG domains [1]. This hybrid molecule was found to bind many intact human Ig molecules and Ig fragments [1]. It proved a powerful tool for the binding, detection and purification of antibodies [2].It was reported that chimeric SpLG was a potent mitogen for mouse splenic B cells, and induced cell differentiation and the production of immunoglobulins. Inhibition experiments demonstrated that the Ig-binding capacity of both SpG and SpL in the chimeric molecule are independent of each other. It was also shown that SpLG selectively absorbed Igs present in the sera of humans, rabbits, mice and rats [1-3]. The preparation of SpLG-HRP by the periodate method is a novel application [4].

References

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MATERIALS

NAME	CATALOG #	VENDOR
Ammonium Sulfate		P212121
Sodium periodate	SB0875.SIZE.100g	Bio Basic Inc.
sodium borohydride	452882	Sigma Aldrich
Horseradish Peroxidase (HRP) type IV	P8375-25KU	Sigma Aldrich
Protein-L from P. Magnus		
Streptococcal protein G by Sigma Aldrich		

MATERIALS TEXT

Pipettes
20ml to 1000 ml glass
Scale
Incubator
Refrigerator
Freezer
Centrifuges

SAFETY WARNINGS

Pay attention to all details as the times of reactions among the proteins involved in this preparation. It will prevent over-oxidation. The average time of preparation is 18 hours.

BEFORE STARTING

All reagents but specially the enzyme and more importantly the sodium periodate solution has to be prepared freshly before mixing it with the enzyme.

1 Horseradish peroxidase (500 µg in 50 µl $NaCO3$, pH 9.6) is mixed with freshly made sodium periodate solution (1.71 mg/ml) followed by incubation in the dark for 2 h.

2 Mix 500 µg of staphylococcal protein-A (SpL) with an equal amount (500 micrograms) of a mix of horseradish peroxidase-sodium periodate. On the other hand mix 500 µg of recombinant protein-L with an equal amount (500 micrograms) of the mix of horseradish peroxidase-sodium periodate.

3 The two mixtures are incubated separately for 3 hours at 4°C with gentle agitation.

4 Forty µl of freshly prepared $NaBH4$ solution (5 mg $NaBH4$ /ml 0.1 mM $NaOH$) is then added separately to the preparations, which are centrifuge (13,000rpm., 10 minutes at RT). Add to each preparation cold saturated amonium sulphate solution and centrifuge again (10000rpm, 25 minutes at 4°C).

5 Now mix the SpL-HRP preparation with SpG-HRP and incubate the mixture for 90 min at 4°C in the dark with gentle agitation.

6 The mixture is then centrifuged for 25 min at 4°C and recover the pellet at the bottom of the tube.

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7 The pellet (SpLG-HRP) is re-suspended in 500 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

8 An equal volume of glycerol is added to the dialysate followed by 200 µl of bovine serum albumin, BSA (20 mg/ ml).

9 The conjugate is then stored at -20°C until further used.

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