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

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1 Works for me dx.doi.org/10.17504/protocols.io.bjkzkkx6

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ABSTRACT

Protein LA, a novel hybrid protein, structurally contains 4 of the Ig Fc-binding and 4 of the Ig Fab binding regions on SpA with 4 of kappa light chain-binding sites of protein L. It has a MW of 65 kDa. Protein LA combines the binding properties of the both SpL and SpA and in some cases, give higher binding affinity than either protein alone. The binding of an Ig to SpL does not interfere with binding of another Ig molecule to the SpA domains and vice versa. Protein LA has been shown to bind effectively to immunoglobulins and their fragments from many species of animals [1].

Reference

1. Justiz-Vaillant AA, Akpaka PE, McFarlane-Anderson N, Smikle MF. Comparison of techniques of detecting immunoglobulin-binding protein reactivity to immunoglobulin produced by different avian and mammalian species. *West Indian Med J.* 2013;62(1):12-20.

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MATERIALS

NAME	CATALOG #	VENDOR
Ammonium Sulfate		P212121
Sodium periodate	SB0875.SIZE.100g	Bio Basic Inc.
sodium borohydride	452882	Sigma Aldrich

NAME	CATALOG #	VENDOR
Horseradish Peroxidase (HRP) type IV	P8375-25KU	Sigma Aldrich
Staphylococcal Protein-A		Sigma Aldrich
Protein-L from P. Magnus		

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 (SpA) 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 mixtures are incubated 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 to the preparation.

5 The preparation (mix of SpA and SpL) is incubated for 90 min at 4°C in the dark with gentle agitation.

6 Cold 50% saturated ammonium sulphate solution (pH 7.4) is added drop by drop in the ratio 1:1 (v/v).

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

8 The pellets is re-suspended in 500 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

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

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

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