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Protocol of preparation of horseradish peroxidase (HRP) conjugated to anti-human IgG to be used as secondary antibody in immunoassays.

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1 Works for me dx.doi.org/10.17504/protocols.io.bjmxkk7n

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ABSTRACT

Peroxidase labeled anti-human IgG is a conjugated secondary antibody that can be used to detect specific antibodies against millions of antigens in humans. This reagent is usually used in enzyme-linked immunosorbent assay, immunoblot analysis and dot blot [1,2].

Reference

1. Stubenrauch K, Wessels U, Essig U, Kowalewsky F, Vogel R, Heinrich J. Characterization of murine anti-human Fab antibodies for use in an immunoassay for generic quantification of human Fab fragments in non-human serum samples including cynomolgus monkey samples.*J Pharm Biomed Anal*. 2013;72:208-215. doi:10.1016/j.jpba.2012.08.023

2.Justiz Vaillant AA, McFarlane-Anderson N, Akpaka PE, Smikle MP, Ramirez N, et al. (2013) Use of Dot Blots Analysis in the Separation of Anti-HIV Antibodies in Animals. J Chromat Separation Techniq 4: 181. doi:10.4172/2157- 7064.1000181

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GUIDELINES

All reagents but specially the enzyme and sodium periodate solution has to be prepared freshly before mixing it with the enzyme. Anti-human IgG can be prepared by immunizing an animal species with purified human IgG.

Then, it can be isolated by a protein purification method and refined by affinity chromatography.

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
ZyMAX™ Goat Anti-Human IgG (H+L) - BT	817140	Thermo Fisher

1 Human IgG can be isolated from human serum or plasma by Protein-A agarose affinity chromatography. To develop the anti-human IgG a laboratory or farm animal is usually immunized and then, the anti-IgG is isolated. The Mancini test can estimate the anti-human IgG concentration.

2 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.

3 Mix 500µg/ml of anti-human IgG with 500 µg of the mix of horseradish peroxidase-sodium periodate. The mixture is 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 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 200 µl of PBS pH=7.4 and dialysed against 1L of PBS for 24 h with 3 buffer changes.

9 An equal volume of glycerol is added to the dialysate followed by 100 µl of bovine serum albumin, BSA (20 mg/ ml).

10 The peroxidase conjugated to anti-human IgG is then stored at -20°C until further used.

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