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Preparation of horseradish peroxidase (HRP) conjugated Streptococcal protein-G by the periodate method.

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ABSTRACT

Streptococcal protein G, type III bacterial Fc receptor, is a globular protein produced by Streptococcal species and is composed of three nearly identical domains, each of 55 amino acids. SpG binds the Fc regions of antibodies IgG from many mammalian species [1-4]. The amino acid sequence of the IgG-binding regions (C1, C2 and C3) of SpG are not homologous to those of the regions (E, D, A, B and C) of SpA. A prerequisite of SpG for successful binding affinity to many IgG is the possession of at least two domains [3].

Streptococcal protein G has been shown to have high binding affinity to sera from various mammalian species including human, , cow, pig, rabbit, goat and sheep by using competitive RIA [5] and to cervids, giraffes and peccaries by direct ELISA [2]. More recently using direct ELISA, some new interactions of SpG with free-ranging nondomestic hoofstock (order Artiodactyla) such as addax, bontebok, antelope, bison, elk, impala, kudu/nyala, oryx, muntjac, sheep, and white-tailed deer have been reported [1,2]. SpG does not bind to the Fc region of the immunoglobulin Y (IgY) of avian species [6]. This reagent can be used in ELISA, Western blotting oand Dot blot to detect antigens and antibodies [1].

It is important in the immunodiagnosis of infectious diseases and other problems. Protein L binds to kappa light chains of immunoglobulins from many animal species including human, mouse, rat, chicken, hamster and pig [1].

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.

2. Stöbel K, Schönberg A, Staak C. A new non-species dependent ELISA for detection of antibodies to Borrelia burgdorferi s. l. in zoo animals. *Int J Med Microbiol*. 2002;291 Suppl 33:88-99. doi:10.1016/s1438-4221(02)80018-2.

3. Kronvall G. A surface component in group A, C, and G streptococci with non-immune reactivity for immunoglobulin G. J Immunol 1973, 111(5): 1401-6.

4. Justiz-Vaillant AA, McFarlane-Andersonv N, Wisdom B, Mohammed W, Vuma S, et al. Immunoglobulin-binding Bacterial Proteins (IBP) Conjugates and their Reactivity with Immunoglobulin in Enzyme-Linked Immunosorbent Assays (ELISA). J Anal Bioanal Tech 2013, 4: 175.

5. Reis KJ, Ayoub EM, Boyle MD. Streptococcal Fc receptors. II. Comparison of the reactivity of a receptor from a group C streptococcus with staphylococcal protein A. J Immunol 1984, 132(6): 3098-102.

6. Serhir B, Dubreuil D, Higgins R, Jacques M. Purification and characterization of a 52-kilodalton immunoglobulin G-binding protein from Streptococcus suis capsular type 2. J Bacteriol 1995, 177(13): 3830-6.

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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
Streptococcal protein G by Sigma Aldrich		

1 Horseradish peroxidase (500 µg in 50 µl $NaCO_3$, 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 protein-G (SpG) with an equal amount (500 micrograms) of a mix of horseradish peroxidase-sodium periodate.

3 The SpG and HRP mixture is incubated for 3 hours at 4°C with gentle agitation.

4 Forty µl of freshly prepared $NaBH_4$ solution (5 mg $NaBH_4$ /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 conjugate 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 HRP conjugated SpG is then stored at -20°C until further used.

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