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Binding properties of Human IgG to immunoglobulin-binding proteins tested by double immunodiffusion (Ouchterlony) technique.

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

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ABSTRACT

1. IBP-HRP conjugates can be prepared in the laboratory by the periodate method.
2. The Human IgG used can be commercially available of prepared in the laboratory from human sera by affinity chromatography (using an SpG-agarose column).
3. SpG-agarose column are widely commercially prepared.
4. PURE-1A antibody purification kit will be discontinued. However there are others product for this purpose as Montage Antibody Purification Kit with PROSEP-G media.

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MATERIALS

NAME	CATALOG #	VENDOR
Affinity Purification Using HiTrap™ Columns		
Montage Antibody Purification Kit with PROSEP-G media		
PURE-1A (antibody purification kit)– Sigma Aldrich		

DISCLAIMER:

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1 The binding of Human IgG to peroxidase-conjugated Immunoglobulin-binding protein (IBP-HRP) including SpA-HRP, SpG-HRP, SpL-HRP, SpLA-HRP, SpAG-HRP, SpLG-HRP, SpLAG-HRP and turtle serum (Sigma-Aldrich) as a negative control are investigated by double immunodiffusion.

2 Briefly, 1% agarose gels are prepared and wells cut into the gel using a template.

3 Initially, aliquots of 25 µl of purified human IgG (Sigma-Aldrich) at a concentration of one (1) µg/µl are applied to the centre well. Human IgG could be prepared homemade by purifying human serum by SpA-affinity chromatography (PURE-1A, Sigma-Aldrich).

4 The peripheral wells are filled with 25 µl each of bacterial protein conjugates including SpA-HRP, SpG-HRP, SpL-HRP, SpLA-HRP, SpAG-HRP, SpLG-HRP, SpLAG-HRP diluted 1:5.

5 The gels are incubated at RT for 48–72 hours incubated in a humidity chamber.

6 After that the gels are examined for precipitin lines.

7 Turtle serum is included as negative controls and all IBP-HRP conjugates suppose to react with human IgG.

8 The positive results are taken as the presence of precipitin line/s and negative results, the absence of precipitin lines.

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