TECHNICAL NOTE

Specificity of KPL's Anti-Human IgG (H+L) Antibody, Peroxidase Labeled, to Human IgG Isotypes

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Understanding the specificity of an antibody to its cognate antigen is essential when designing and optimizing an immunoassay. Affinity purified polyclonal antibodies are an excellent choice for most immunoassays, as they provide better specificity than other antibodies purified by Protein A/G or unfractionated antibodies. Antibodies made against IgG may have variable isotype specificities. Ideally, a secondary antibody will detect all IgG isotypes equally. However, isotype coverage is not always known or may not be complete when using commercially available secondary antibodies. Knowledge of reactivity to specific isotypes allows researchers to select the best antibody for their assays.

IgG Isotypes

IgG consists of 2 identical heavy chains (~50 kDa) and 2 identical light chains (~25 kDa). The heavy chain in human IgG also is called the gamma chain (γ). Human IgG can be subdivided into 4 subclasses based on slight variations in the amino acid sequence of the heavy (gamma) chain: IgG1, IgG2, IgG3, and IgG4. In addition to the subclasses, antibodies have one of 2 types of light chains: kappa (κ) and lambda (λ). All together, there are eight human IgG isotypes: IgG1 κ, IgG1 λ, IgG2 κ, IgG2 λ, IgG3 κ, IgG3 λ, IgG4 κ, and IgG4 λ.

The concentration of IgG isotypes in serum varies depending on isotype expression levels and health status of the individual. Typically in healthy humans, the levels of IgG isotypes are approximately: IgG1 (66%), IgG2 (24%), IgG3 (7%), and IgG4 (3%)[1]. Additionally, the kappa-to-lambda ratio in healthy humans is about 2:1.

Scope

KPL's Anti-Human IgG (H+L) Antibody, Peroxidase Labeled (KPL Catalog No. 074-1006), was examined for the ability to bind to all 4 isotypes, as well as their requisite light chains.

Human Isotypes

Isotype	Sigma Catalog No.	Lot No.
IgG1 κ	I5154	051M6257
IgG2 κ	I5404	067K6026
IgG3 κ	I5654	011M6157
IgG4 κ	I4639	021M6055
IgG1 λ	I5029	091M6298
IgG2 λ	I5279	061M6247
IgG3 λ	I5529	061M6280
IgG4 λ	I4764	060M6149

Polyclonal Secondary Antibodies

Anti-Human IgG (H+L) Antibody, Peroxidase Labeled (rehydrated to 1.0 mg/mL in 50% glycerol; KPL Catalog No. 074-1006, Lot No. 100609)

Western Blot Protocol

1. Fifty nanograms of each IgG isotype were separated by reducing SDS-PAGE and transferred onto a nitrocellulose membrane.

2. The immunoblot was blocked in 25 mL of 5X Detector Block (diluted 1:5 in reagent quality water), with 1% Detector Block Powder (KPL Catalog No. 71-83-00) for 2 hours with shaking.

3. The immunoblot was probed with Anti-Human IgG (H+L) Antibody, Peroxidase Labeled, in 5X Detector Block (diluted 1:5 in reagent quality water) at a concentration of 1 µg/mL for 1 hour with shaking.

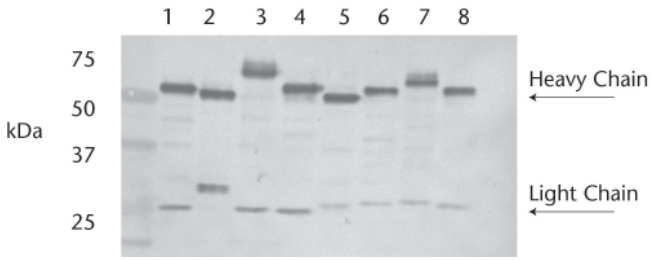
4. The immunoblot was washed in 25 mL of 10X Tris Buffered Saline with 0.5% Tween-20 (diluted 1:10 in reagent quality water; KPL Catalog No. 51-18-01) once for 15 minutes, and then 3 times more for 5 minutes each.

5. The immunoblot was rinsed in 25 mL of reagent quality water for 5 minutes.

6. The immunoblot was developed with TMB 1-Component Membrane Peroxidase Substrate (KPL Catalog No. 50-77-03) for 1 minute.

7. The blot was rinsed with reagent quality water, blotted dry, and imaged.

RESULTS



Lane	Isotype
1	Human IgG1 κ
2	Human IgG2 κ
3	Human IgG3 κ
4	Human IgG4 κ
5	Human IgG1 λ
6	Human IgG2 λ
7	Human IgG3 λ
8	Human IgG4 λ

SUMMARY

KPL's Anti-Human IgG (H+L) Antibody, Peroxidase Labeled, detects all human isotypes including both heavy and light chains. Kappa light chain detection is stronger than lambda light chain possibly reflecting the 2:1 abundance of kappa to lambda chains in IgGs.

Knowledge of antibody isotype specificity is important when performing various immunosassays. KPL's affinity purified antibody, which detects all human IgG isotypes, makes an excellent secondary antibody conjugate when broad isotype specificity is required.

REFERENCES

1. Pan, Q. and Hammarström, L. Molecular Basis of IgG Subclass Deficiency. Immunological Reviews 2000;178:99-110.

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