

## KPL TECHNICAL NOTE

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

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Affinity purified polyclonal antibodies are an excellent choice in most immunoassays, as they provide better specificity than other antibody forms (e.g., whole serum or Protein A/Protein G purified). Polyclonal antibodies made against IgG may have variable isotype specificities due to the heterogeneous nature of IgG isotypes. In most immunoassay applications, 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 mouse IgG is also called the gamma chain ( $\gamma$ ). Mouse IgG can be subdivided into 4 subclasses based on slight variations in the amino acid sequence of the heavy (gamma) chains: IgG1, IgG2a, IgG2b, and IgG3. In addition to the subclasses, IgG has 2 types of light chains: kappa ( $\kappa$ ) and lambda ( $\lambda$ ). Thus there are 8 mouse IgG isotypes: IgG1  $\kappa$ , IgG1  $\lambda$ , IgG2a  $\kappa$ , IgG2a  $\lambda$ , IgG2b  $\kappa$ , IgG2b  $\lambda$ , IgG3  $\kappa$  and IgG3  $\lambda$ .

The concentrations of IgG isotypes in serum may differ depending on variables such as isotype expression levels, health status, species, and age of the mouse. In healthy Balb/C mice the levels of IgG isotypes ( $\kappa$  and  $\lambda$  combined) are approximately: IgG1 (17%), IgG2a (39%), IgG2b (29%), and IgG3 (16%)<sup>1</sup>.

### Scope

KPL's Anti-Mouse IgG (H+L) Antibody, Human Serum Adsorbed (HSA) and Peroxidase Labeled (KPL Catalog No. 074-1806), was examined for the ability to bind to all 4 isotypes, and their requisite light chains.

### Mouse Isotypes

Isotype	Sigma Catalog No.	Lot
IgG1 ( $\kappa$ & $\lambda$ )	M5284	060M4764
IgG2a ( $\kappa$ & $\lambda$ )	M5409	041M4766
IgG2b ( $\kappa$ & $\lambda$ )	M5534	061M4817
IgG3 $\kappa$	I3784	110M6112
IgG3 $\lambda$	M9019	021M4775

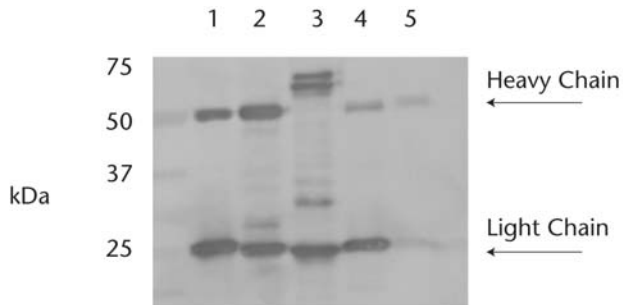
### Polyclonal Secondary Antibodies

Anti-Mouse IgG (H+L) Antibody, HSA and Peroxidase Labeled (rehydrated to 1.0 mg/mL in 50% glycerol; KPL Catalog No. 074-1806, Lot# 110375)

### 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-Mouse IgG (H+L) Antibody, HSA and Peroxidase Labeled, in 5X Detector Block (diluted 1:5 in reagent quality water) at a concentration of 1  $\mu$ g/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	Mouse IgG1 ( $\kappa + \lambda$ )
2	Mouse IgG2a ( $\kappa + \lambda$ )
3	Mouse IgG2b ( $\kappa + \lambda$ )
4	Mouse IgG3 $\kappa$
5	Mouse IgG3 $\lambda$

## SUMMARY

KPL's Anti-Mouse IgG (H+L) Antibody, HSA and Peroxidase Labeled, detected all mouse isotypes, with both heavy and light chains visible on the blot. Natural low abundance of IgG3 lambda may account for fainter bands seen on the Western blot. Dividing IgG3 into kappa and lambda components may also account for a weaker signal.

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

## REFERENCES

1. McKendall, RR and Woo, W. Murine IgG Subclass Responses to Herpes Simplex Virus Type 1 and Polypeptides. J. Gen. Virol. 1988: 69:847-857.



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