antibodies

Supplementary: Binding of Immunoglobulin G to Protoporphyrin IX and Its Derivatives: Evidence the Fab Domain Recognizes the Protoporphyrin Ring

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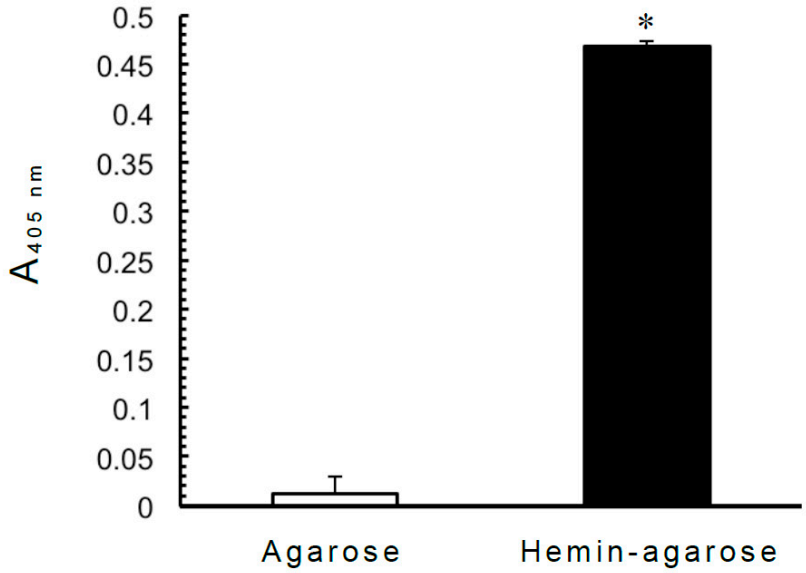

Figure 1. Binding of ALP-labeled anti-bovine Tf antibodies to hemin-agarose beads. Hemin-agarose and agarose were purchased from Sigma Chemical Co. (St. Louis, MO, USA). A 1-mL volume of PBS containing ALP-conjugated anti-Tf antibodies (25 μg), prepared according to a previously described method (Orino et al. 1993; Watanabe et al. 1994), and hemin-agarose beads or agarose beads in PBS (net volume of beads per sample: 25 μL each) was added to a Spitz glass tube masked with 0.1% (*w*/*v*) BA. The mixture was centrifuged at 1700× *g* for 15 min, and the pelleted beads were then washed three times with 1 mL of PBS. After washing, the pelleted beads were re-suspended with 1 mL of 3 mM disodium *p*-nitrophenyl phosphate. After incubation at 37 °C, the mixture was centrifuged at 1700× *g* for 5 min at room temperature, and the absorbance of the resulting supernatant was measured at 405 nm. Each value is the mean ± SD of three tests. * $p < 0.01$ versus binding examined with agarose beads.