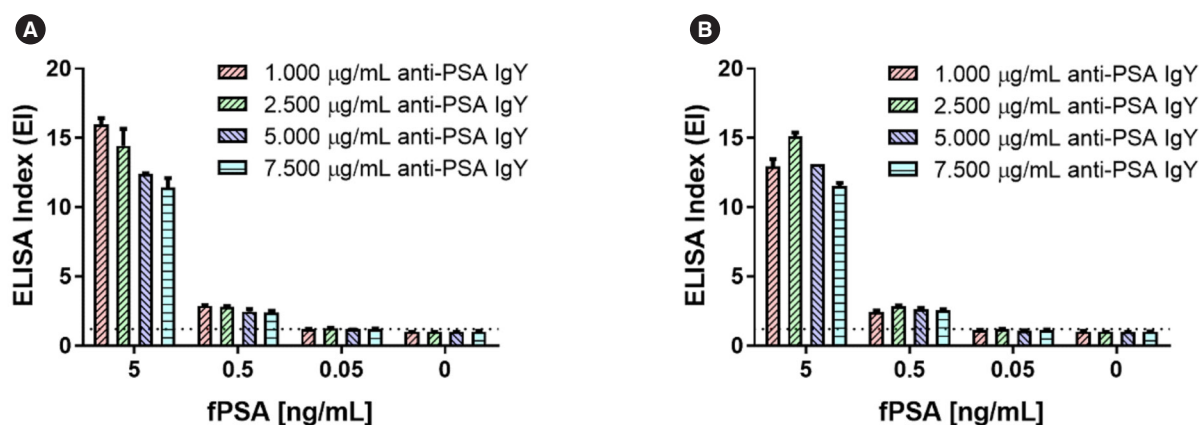


SUPPLEMENTAL MATERIALS

Optimization of the IgY-Based ELISA

Optimization of the IgY-based ELISA for PSA detection was performed for different concentrations of PSA-specific mouse monoclonal IgG and avian polyclonal IgY antibodies (Supplemental Data Fig. S1). The microtiter plate was coated with an anti-PSA mouse monoclonal IgG antibody, blocked, and incubated with fPSA at different concentrations (5.000, 0.500, and 0.050 ng/mL). For the detection of an antigen, affinity purified anti-PSA IgY antibodies were used at different concentrations (1.000, 2.500, 5.000, and 7.500 $\mu\text{g/mL}$). The results are presented as $\text{EI} \pm \text{SEM}$ values. As optimal conditions, we assumed both antibody concentrations were 2.500 $\mu\text{g/mL}$ because of the highest ELISA Index (where $\text{EI} = \text{OD}_{\text{sample}} / \text{OD}_{\text{control}}$). Values of > 1.200 were considered positive [36] for 50.000 $\mu\text{g/mL}$ of PSA protein, which was determined as the detection limit, as previously shown [16].



Supplemental Data Fig. S1. Optimization of IgY-based sandwich ELISA for two different concentrations of IgY antibodies (A. 2.500 $\mu\text{g/mL}$, B. 5.000 $\mu\text{g/mL}$).

Abbreviation: fPSA, free prostate specific antigen.