To determine possible differences in the tPSA concentration values, which might result from the use of a particular assay buffer, we performed ELISA using samples with a tPSA concentration within the linear range of our IgY-based ELISA (slightly over 2,000 ng/mL), prepared/diluted in an assay buffer and in commercial calibrator serum without PSA protein (Cal1) and undiluted. The microtiter plate was coated with an anti-PSA mouse monoclonal IgG antibody (2,500 µg/mL), blocked, and incubated with different serum samples (undiluted and at a concentration of 1,000, 500, and 50 ng/mL). The exact concentrations of tPSA (REF values) are marked above the x axis. For detection of the antigen, an affinity purified anti-PSA IgY antibody was used (2,500 µg/mL). The results are presented as OD$_{490}$ ± SD values.

Based on the obtained absorbance values, we determined and compared linear regression curves (Supplemental Data Fig. S3).

In addition, we determined the accuracy and precision of two samples that were prepared as Cal2 dilutions in assay buffer (2,250 and 0,250 ng/mL) among all 16 ELISA plates. The percent difference was 2.640% for the 2,250 ng/mL sample and 3.850% for the 0.250 ng/mL sample. The precision, determined as the range between measurements, was 0.140 and 0.090 ng/mL, respectively.

Supplemental Data Fig. S4. Matrix evaluation in an IgY-based ELISA for different samples. Abbreviations: PSA, prostate specific antigen; PSA154, PSA166, PSA 190, serum samples; Cal0, commercial calibrator; BSA, bovine serum albumin; OD, optical density.