## Performance of the IgY-Based ELISA Assay

To determine whether the samples with a PSA protein concentration above the limit of quantification can be diluted into the valid range for analysis, we performed dilutional linearity evaluation with a tPSA ELECSYS calibrator (Cal2, 64.900 ng/mL; Roche Diagnostics).

The microtiter plate was coated with an anti-PSA mouse monoclonal IgG antibody (2.500 µg/mL), blocked, and incubated with a commercial calibrator sample (64.900 ng/mL), serial dilutions (50.000–0.050 ng/mL) of the calibrator, a selected serum sample (62.730 ng/mL), and serial dilutions of the serum sample ranging from 50.000 to 0.050 ng/mL. To detect the antigen, an affinity purified anti-PSA IgY antibody was used (2.500 µg/mL). The results are presented as $OD_{490} \pm SD$ values.

Unfortunately, because of the calibrator concentration, we were unable to reach a concentration high enough to examine the presence of the Hook Effect, and the possibility of diluting the sample was limited (Supplemental Data Fig. S3). As a result, samples diluted from the Cal2 64.900 ng/mL with assay buffer (0.100% BSA in PBS) or with the tPSA ELECSYS calibrator "0" (Cal1; Roche Diagnostics) allowed us to obtain similar values for samples diluted to 5.000 and 0.500 ng/mL. The ratios between determined and expected values were 1.230 and 1.180, respectively, when calculated from the 4PL curve (for linear regression equation, the values were 1.260 and 1.210, respectively). For the serum sample selected according to the criterion of similar concentration (62.730 ng/mL), the ratio of the sample and the commercial Cal2 (64.900 ng/mL) was equal to 1.110 from the 4PL curve (1.200 when calculated from the linear regression equation).
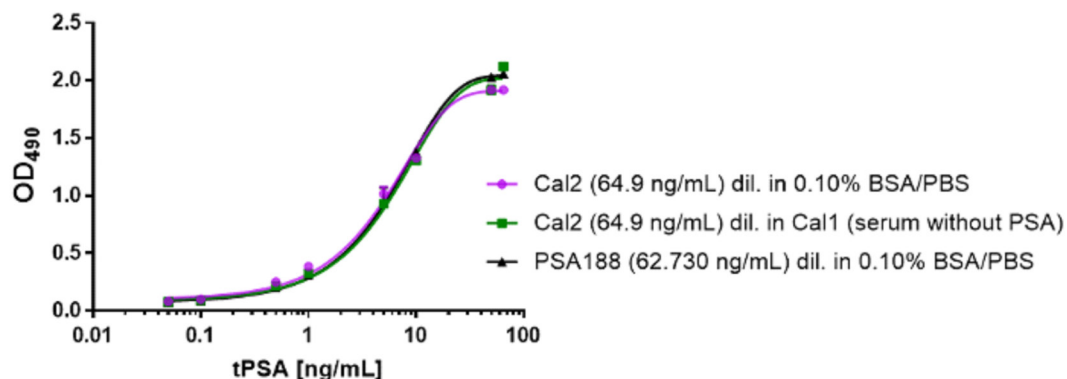
Supplemental Data Fig. S3. Dose-response curve in a IgY-based ELISA for different samples and dilution agents.
Abbreviations: BSA, bovine serum albumin; PSA, prostate specific antigen; tPSA, total PSA; Cal1/Cal2, commercial tPSA calibrators; OD, optical density.