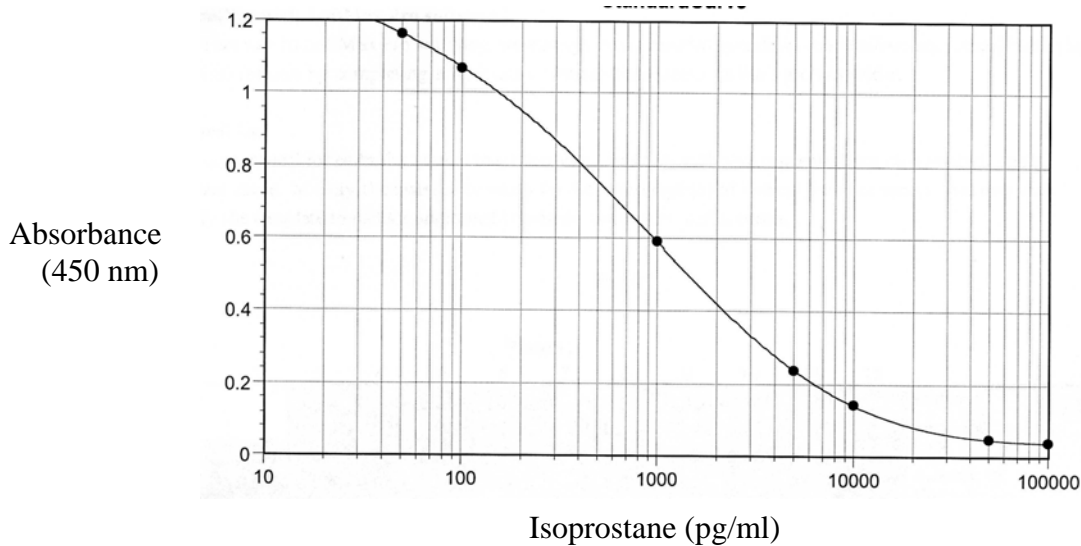


## Urinary Isoprostane EIA Analysis



Urine Required: 300  $\mu$ l  
Range of Values Observed: 1 – 18 ng/mg creatinine

15-isoprostane  $F_{2t}$  is measured utilizing a competitive enzyme-linked immunosorbent assay (ELISA) kit from Oxford Biomedical Research, Inc., Oxford, MI (Catalogue # EA85). Urine samples are thawed and mixed with 4  $\mu$ L glucuronidase (250,000 units/mL), Oxford Biomedical Research, Inc, Oxford, MI (Catalogue # GL85) and incubated for two hours @ 37 °C and then centrifuged for 2 minutes at 2,000 rpm in a microfuge. Standards and samples (100  $\mu$ l) are added in duplicate to 96 well plates, followed by addition of 100  $\mu$ L of diluted  $F_{2t}$  HRP conjugate and incubated for two hours at room temperature. After washing to remove any unbound substances 200  $\mu$ L of substrate solution is added to each well and color allowed to develop proportionate to the amount of isoprostane present. The color development is stopped with the addition of 50  $\mu$ L 3N  $H_2SO_4$  and the microplate then read at 450 nm and also at 590 nm as a background control. Plots of log concentration vs absorbance for standards are prepared and concentrations of unknown samples extrapolated from the standard curve using a four parameter fit and adjusted for any dilution of urine and reported as pg/mL. After measurement of urinary creatinine, isoprostane is then calculated and reported as ng/mg creatinine.