

PROSTATE-IC

code XG007

Biomarker	Sens	Spec	PPV	NPV
PSA-IgM (145,1 AU/mL)	40%	88%	77%	60%
PSA (4 ng/mL)	84%	4%	46%	20%
PSA (10 ng/mL)	22%	71%	42%	48%
PSA (10 ng/mL) o PSA-IgM (145,1 AU/mL)	60%	63%	61%	62%
PSA (>4 e <10 ng/mL) e PSA-IgM (145,1 AU/mL)	47%	88%	76%	55%

Table 1: Comparison of specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) of PSA-IgM and PSA in differentiating patients with prostate cancer (n = 50) from patients with benign prostatic hyperplasia (n = 51).

REFERECES

1. Beneduce *et al.* Annual Congress EAU, Paris, April 5–8, 2006
2. Beneduce *et al.* Cancer Detect Prev 2007, 31 (5):402-7
3. Prayer-Galetti *et al.* Annual Congress SIU, Bologna, June 17- 21, 2006
4. Prayer-Galetti *et al.* Annual Congress SIU, Bari, September 27 - October 1, 2007.
5. Zani *et al.* Annual Congress SIU, Roma, September 22-28, 2008
6. Zani *et al.* Annual Congress SIU, Rimini, October 4-7, 2009

REAGENTS AND MATERIALS PROVIDED

XG007-PL: 96 wells multi-strip Assay-Plate, precoated with affinity purified rabbit anti-PSA.

XG007-Calibrator: Two vials of calibrator lyophilized from PBS. White powder. Exact concentration on label. Totally soluble.

XG-EA: 200 µL of Enzyme-conjugated goat anti-human IgM secondary antibody (Green cap) 100-fold concentrate solution in PBS containing 1% BSA.

XG-CH3: 10 mL of TMB (3,3',5,5'-Tetramethylbenzidine) chromogen solution ready to use.

XG-ST3: 10 mL of 1N HCl Stop solution ready to use.

XG-DB5: 10 mL of 5X concentrated Dilution Buffer solution.

XG-WB2: Two tablets of lyophilized Washing Buffer. White powder. Once diluted, the working solution contains 0.05% Tween 20 in PBS. Totally soluble.

MATERIAL AND EQUIPMENT REQUIRED

Precision pipettes with disposable tips

Microplate washer

Microplate readers with a 450 ± 20 or 650 ± 20 nm filter

Distilled or deionized water

BRIEF DESCRIPTION OF PROCEDURE

Calibration curve and samples:

Reconstitute XG007-Calibrator with 440 L of distilled water for each calibrator vial. Dispense 100 µL/well of standard calibrators (in duplicate) starting from the reconstituted solution and performing in-plate 2-fold serial dilutions in order to obtain a seven-point calibration curve. Use XG-DB5 dilution buffer as diluent. For exact concentration of the reconstituted calibrator please refer to the concentration value (AU/mL) indicated on the XG007-Calibrator vial. Dispense 100 µL/well of a 50- or 100-fold diluted sample (in duplicate). Use XG-DB5 dilution buffer as diluent. Also dispense 100 µL/well of XG-DB5 dilution buffer as blank, in duplicate. Incubate 1

hour at room temperature. Wash 6x with XG-WB2 washing buffer (300 µL/well).

Secondary antibody:

Add 100 µL/well of diluted XG-EA enzyme-conjugated secondary antibody solution. Incubate 1 hour at room temperature. Wash six times with XG-WB2 washing buffer (300 µL/well).

TMB Substrate solution:

Apply 100 µL/well of XG-CH3 chromogen solution. Allow color to develop for 10-15 min at room temperature in the dark and measure OD values of each well using an ELISA plate reader with a 650 nm filter or, alternatively, apply 100 µL/well of XG-ST3 Stop Solution and measure OD values of each well using an ELISA plate reader equipped with a 450 nm filter. Stopped reaction should be read within 1 hour.

PROCESSING OF THE RESULTS

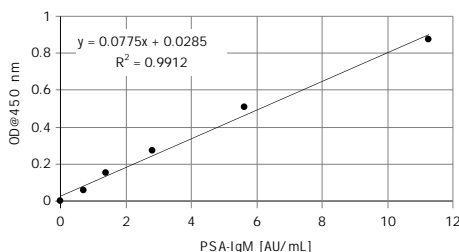


Figure 3A: range of linearity (0 to 11.25 AU/mL) of a typical standard curve for PSA-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

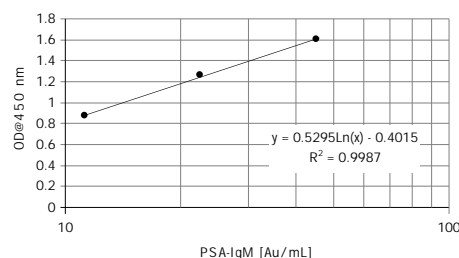


Figure 3B: range of linearity (form 11.25 to 45.0 AU/mL) of a typical semi-logarithmic standard curve for PSA-IgM after 15 minutes of substrate incubation at room temperature and addition of stop solution

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