



DIAGNOSTIC AUTOMATION, INC.

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See external label  2°C-8°C



Σ=96 tests



#4227-11

MICROWELL ELISA

Prostatic Acid Phosphatase PAP

SUMMARY OF ASSAY PROCEDURE

| Step | (20-25°C Room temp.) | Volume | Incubation time |
|------|----------------------------|--------|-----------------|
| 1 | Samples & Calibrators | 50 µl | |
| 2 | Sample diluent | 50 µl | 30 minutes |
| 3 | Wash 3 times with DI water | 350 µl | |
| 4 | Enzyme conjugate | 100 µl | 30 minutes |
| 5 | Wash 3 times with DI water | 350 µl | |
| 6 | TMB Chromogenic Substrate | 100 µl | 30 minutes |
| 7 | Stop solution | 100 µl | |
| 8 | Reading OD 450 nm | | |

INTENDED USE

For the quantitative measurement of human prostatic acid phosphatase (PAP) in human serum and plasma.

SUMMARY AND EXPLANATION OF TEST

Prostatic acid phosphatase (PAP) enzyme activity was first measured in the urine of men and was found to be localized in organs of the male genital tract. Gutman and co-workers suggested that PAP may be a significant tumor marker in those patients with prostate cancer because serum PAP concentrations were found to be elevated in many men with primary prostatic carcinoma and metastatic lesions of prostate.

In 1938, Gutman and Gutman reported elevated serum acid phosphatase activity in prostatic cancer patients, especially those with bone metastasis. Subsequent studies confirmed that this increased enzyme activity was of prostatic origin; also, the properties of this prostatic enzyme differed from those of acid phosphatase in normal serum.

For many years serum acid phosphatase has been measured by spectrophotometric assays based on enzyme activity. These colorimetric methods utilize various substrates, some in conjunction with differential inhibitors of prostatic acid phosphatase. Generally, these assays lack sensitivity or specificity; also, the stability of serum enzymatic activity is time, temperature, and pH dependent. ELISA has been developed to provide a method of high sensitivity and specificity.

PRINCIPLE OF THE PROCEDURE

DIAGNOSTIC AUTOMATION ELISA PAP is a microwell enzyme linked immunosorbent assay based on the sandwich principle. The wells are coated with anti-PAP antibodies. The samples and Standards are incubated in coated wells. During incubation, if antigen PAP is present, a complex is formed on the well. Unbound substances are washed off. Enzyme conjugate then is added to form a sandwich complex. Unbound substances are washed off again. TMB Chromogenic Substrate is added to develop a color. The enzymatic reaction is

stopped by the addition of acid and the intensity of color developed is read using a microwell reader at 450 nm. The PAP in specimens run concurrently with standards can be determined from the standard curve.

MATERIALS PROVIDED

1. Microwell Strips: Anti-PAP antibodies coated wells. (12 strips x 8 wells)
2. TMB Chromogenic Substrate: Amber bottle. 1 vial (15 ml)
3. Enzyme conjugate: Red color solution. 1 vial (12 ml)
Anti-PAP antibodies conjugated to horseradish peroxidase.
4. Sample Diluent: 1 vial (12 ml)
5. Standard Set: 0, 1.5, 5, 15, 30, 60 ng / ml (1.0 ml / vial)
6. Stop Solution: 2 N HCl. 1 vial (12 ml)

SPECIMEN COLLECTION AND HANDLING

1. Collect blood specimens and separate the serum.
2. Specimen may be refrigerated at 2-8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing of serum samples.

WARNINGS AND PRECAUTIONS

1. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
2. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. It is recommended to use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

PREPARATION FOR ASSAY

1. Bring all reagents and specimens to room temperature (20-25 °C) and gently swirl to obtain thorough mixing.
2. Have all reagents and samples ready before the start of the assay. Once the test is begun, it must be performed without interruption to get the most reliable and consistent results.

ASSAY PROCEDURE

**50 + 50 / 100 / 100
30 / 30 / 30 RT**

1. Place the desired number of coated strips into the holder.
2. Dispense 50 µl of standards and specimens into the appropriate wells.
3. Dispense 50 µl of Sample diluent to each well and incubate for 30 minutes at room temperature.
4. Remove incubation solution and wash three times with distilled water or tap water.
5. Dispense 100 µl of enzyme conjugate, to each well and incubate for 30 minutes at room temperature.
6. Remove incubation solution and wash three times with distilled water or tap water.
7. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 N HCl to stop reaction.
9. Read O.D. at 450 nm with a microwell reader.

PROCEDURE NOTE

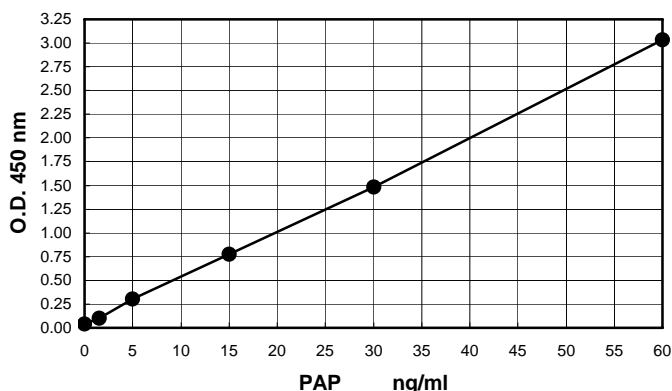
1. It is important to wash microwells thoroughly and remove the last droplets of water to achieve the best results.
2. Pipette all reagents and samples into the bottom of the wells.
3. Absorbance is a function of time and temperature of incubations. It is recommended to have all reagents and sample caps removed and all needed

wells assigned and secured in holder. It will ensure the equal elapsed time for each pipetting without interruption.

CALCULATION OF RESULTS

- Construct a standard curve by plotting O.D. 450 nm on the y-axis against the concentration of PAP ng/ml on the x-axis using linear graph paper or log-log graph paper.
- Using the O.D. value of each specimen, determine the concentration of PAP from the standard curve.
- An example of typical results:

| Standard Set | PAP (ng/ml) | O.D. 450 nm | | O.D. 450 nm Mean | SD | CV % |
|-------------------|-------------|-------------|-------|------------------|-------|-------|
| Standard 1 | 0 | 0.046 | 0.041 | 0.044 | 0.004 | 8.128 |
| Standard 2 | 1.5 | 0.108 | 0.099 | 0.104 | 0.006 | 6.149 |
| Standard 3 | 5 | 0.302 | 0.306 | 0.304 | 0.003 | 0.930 |
| Standard 4 | 15 | 0.773 | 0.778 | 0.776 | 0.004 | 0.456 |
| Standard 5 | 30 | 1.501 | 1.464 | 1.483 | 0.026 | 1.765 |
| Standard 6 | 60 | 3.003 | 3.059 | 3.031 | 0.040 | 1.306 |
| Control 1 - 40121 | 1.740 | 0.116 | 0.113 | 0.115 | 0.002 | 1.853 |
| Control 2 - 40122 | 27.591 | 1.232 | 1.229 | 1.231 | 0.002 | 0.172 |



EXPECTED VALUES

- It is recommended that each laboratory determine its own normal value and abnormal range.
- The results of a clinical study using DIAGNOSTIC AUTOMATION ELISA PAP were summarized:
 - Serum samples from 95 normal subjects were assayed. In this population, 99% of the values was less than 3 ng/ml.
 - Samples from 69 patients with benign prostatic hypertrophy (BPH) were assayed and found 94% less than 3 ng/ml.
 - Samples from 43 patients with prostatic carcinoma (PC) were assayed and found 81% higher than 3 ng/ml.

PERFORMANCE CHARACTERISTICS

Accuracy (Recovery)

Recovery studies were performed by mixing an aliquot of pooled serum and PAP standard. The PAP values were measured and percentage of recovery were determined.

| | Initial values ng/ml | Conc. Spiked ng/ml | Expected values ng/ml | Observed values ng/ml | Recovery % |
|----|----------------------|--------------------|-----------------------|-----------------------|------------|
| A: | 4 | 5 | 4.5 | 4.5 | 100 |
| | 4 | 15 | 9.5 | 10.0 | 105 |
| | 4 | 30 | 17.0 | 16.8 | 99 |
| B: | 10.8 | 5 | 7.9 | 8.3 | 105 |
| | 10.8 | 15 | 12.9 | 13.0 | 101 |
| | 10.8 | 30 | 20.4 | 20.5 | 99 |
| C: | 28 | 3 | 15.5 | 15.0 | 97 |
| | 28 | 5 | 16.5 | 17.0 | 103 |
| | 28 | 15 | 21.5 | 23.5 | 109 |

Parallelism

| Dilution | Calculated (ng/ml) | Observed (ng/ml) | Recovery % |
|----------|--------------------|------------------|------------|
| 4 in 4 | | 86.0 | |
| 3 in 4 | 64.5 | 69.7 | 108 |
| 2 in 4 | 43.0 | 45.2 | 105 |
| 1 in 4 | 21.5 | 21.0 | 98 |

Precision (Reproducibility)

Inter-assay (n=12) and intra-assay (n=12), coefficient of variation, were evaluated at three different pooled serum samples:

| | Inter-assay | | | Intra-assay | | |
|--------------|-------------|--------|--------|-------------|--------|--------|
| | Pool A | Pool B | Pool C | Pool A | Pool B | Pool C |
| N | 12 | 12 | 12 | 12 | 12 | 12 |
| Mean (ng/ml) | 3.73 | 9.91 | 18.7 | 5.62 | 11.21 | 22.75 |
| S.D. (ng/ml) | 0.20 | 0.98 | 0.96 | 0.23 | 0.62 | 0.94 |
| C.V. % | 5.28 | 9.92 | 5.15 | 4.15 | 5.57 | 4.14 |

Sensitivity (Minimum detectable concentration)

The minimal detectable concentration of PAP is estimated to be 0.2 ng/ml. The minimum detectable concentration is defined as that concentration of PAP which corresponds to the absorbance value that is two standard deviations greater than the mean absorbance value of twenty replicate determinations of the sample diluent.

Specificity (Cross-reactivity)

The following human hormones and tumor markers were tested for cross-reactivity of this assay:

| Tested hormones or tumor markers | Amount | Produced Color Intensity Equivalent to PAP (ng/ml) |
|----------------------------------|-----------|--|
| Human TSH | 25 uIU/ml | undetectable |
| Human AFP | 100 IU/ml | undetectable |
| Human CEA | 30 ng/ml | undetectable |

LIMITATIONS

1. The PAP values should be used as an adjunct to other data available to the physician.
2. Sample with PAP level above 60 ng/ml should be diluted to obtain an accurate value.

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