Acid Phosphatase (ACP)  
(Colorimetric Test with α-Naphthylphosphate)  

R1 Substrate  5 x 10 ml  
R2 For Total ACP  1 x 50 ml  
R3 For Non-Prostatic ACP  1 x 50 ml  
R4 Stabilizer  1 x 2 ml  

METHOD / REACTION PRINCIPLE:  
α-Naphthylphosphate is hydrolysed by ACP to phosphate and α-naphthol, which is converted with FRTR-salt into an azo dye. The increase of absorbance at 405 nm is proportional to the total ACP activity in the sample. The prostatic acid phosphatase (PACP) can be blocked by tartrate and can be determined indirectly (through the non-prostatic ACP) by calculation of the activity difference.  

\[
\begin{align*}
\alpha\text{-Naphthylphosphate} + H_2O & \rightarrow \text{ACP} \rightarrow \text{phosphate} + \alpha\text{-naphthol} \\
\alpha\text{-Naphthol} + \text{FRTR-salt} & \rightarrow \text{Azo dye}
\end{align*}
\]

REAGENTS: (Concentrations in the test)  
R1 : 1-Naphthyl phosphate 10 mmol/l  
Fast Red TR-salt 1.5 mmol/l (4-chloro-2-methylphenyl diazonium salt)  
R2 : Citrate buffer pH 5.2 100 mmol/l  
R3 : Citrate buffer pH 5.2 100 mmol/l  
Tartrate 135 mmol/l  
R4 : Stabilizer (Acetic acid) 0.8 mol/l  

The sealed reagents are stable up to the indicated expiry date if stored at 2° - 8°C.  

PREPARATION AND STABILITY OF WORKING REAGENTS  
R2 and R3 are ready for use  
Reagent A (determination of Total ACP):  
Dissolve the contents of R1 (substrate) in 10 ml of buffer solution R2.  
Mark label with „A“.  
Reagent B (determination of Non-Prostatic ACP):  
Dissolve the contents of R1 (substrate) in 10 ml of tartrate solution R3. Mark label with B  

Stability of Working reagents  
3 days at 2° - 8°C  
1 day at 18° - 25°C  

SAMPLES:  
Serum, no plasma! Avoid hemolysis!  
Use immediately or stabilize:  
Add 1 drop of 0.1% acetic acid to 1 ml of serum:  
ACP is stable for 3 days at 2-8°C.  

PROCEDURE:  
I-Manual assay for Total ACP  
Reagent A  1.0 ml (Pre-heated At 37°C)  
Sample  100 µl  
Mix, Wait 5 minutes then measure absorbance (at 405 nm) each one minute for 3 minutes. Determine \( \Delta A /\text{min} \)  
Calculation:  \( \Delta A /\text{min} \times 743 = \text{TACP Activity in sample} \)  

II-Manual assay for Non-Prostatic ACP  
Reagent B  1.0 ml (Pre-heated At 37°C)  
Sample  100 µl  
Mix, Wait 5 minutes then measure absorbance (at 405 nm) each one minute for 3 minutes. Determine \( \Delta A /\text{min} \)  
Calculation:  \( \Delta A /\text{min} \times 743 = \text{NPACP Activity} \)  

CALIBRATORS AND CONTROLS  
For the calibration of automated analyzers Spectrum Multicalibrator is recommended, for quality control use Spectrum Normotrol and pathotrol.  

LINEARITY:  
The reaction is linear up to ACP concentration 74 U/L, Specimen Shows higher concentration should be diluted 1+3 with physiological saline and repeat assay (Result x 3).  

Expected Values  
Total Acid Phosphatase  
Men  \( \leq 4.7 \) U/l  
Women  \( \leq 3.7 \) U/l  
Prostatic Acid Phosphatase  \( \leq 1.6 \) U/l  

LITERATURE  