Alkaline Phosphatase (ALP)
Alkaline Phosphatase

- Alkaline phosphatase is found in liver, bone, intestine, placenta and kidney, the main sources of serum enzyme are the hepatobiliary tree and osteoblasts.

- Physiologically increased levels are seen during periods of active bone growth, particularly in infants and at puberty.
Alkaline Phosphatase

- Values increase during the second and third trimesters of pregnancy to about twice those normally seen in adults.

- Pathologically increases in serum alkaline phosphatase occur mainly in hepatobiliary disease and bone disease.
Alkaline Phosphatase

- Alkaline phosphatase activity in serum is usually estimated to detect increased levels, but markedly reduced levels are found in the inherited condition (hypophosphatasia), which is caused by defective bone calcification.
Alkaline Phosphatase

-To identify the tissue of origin by determining alkaline phosphatase isoenzymes or by estimating the activity of another enzyme, usually Gamma-glutamyl transferase, which rises in parallel with biliary alkaline phosphatase.
Alkaline Phosphatase

• ALP is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides and proteins.

• Most effective in an alkaline environment

• In humans, alkaline phosphatase is present in all tissues throughout the entire body, but is particularly concentrated in liver, bile duct, kidney, bone, and the placenta.

• Levels are significantly higher in children and pregnant women.
Alkaline Phosphatase

• Abnormally high blood levels of ALP may indicate two groups of conditions:
  - Hepatobiliary disease: bile duct obstruction
  - Bone disease associated with increased osteoblastic activity: Paget's disease (*a chronic disorder that typically results in enlarged and deformed bones*), osteogenic sarcoma, rickets.

• Abnormally low levels of ALP is a genetic condition called hypophosphatasia which results in bone deformities.
Paget's disease

Rickets:
Before treatment (Bowleg deformity) and 2 years after treatment with calcium
Alkaline Phosphatase
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  - the rate of appearance of product or the rate of disappearance of substrate
• \([S]: \) more than \([E]\)

• Salt Concentration
  
  • Most enzymes cannot tolerate extremely high salt concentrations. Typical enzymes are active in salt concentrations of 1-500 mM.

• optimum temperature

• optimum pH

• cofactor
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• **1 unit (U):** the amount of enzyme that catalyses the reaction of 1µmol of substrate per minute under standard conditions (International unit).

• In clinical lab, the unit of the enzyme activity is usually expressed in a traditional way as the detection method.
• **Under alkaline conditions**, ALP catalyse the hydrolysis of disodium phenyl phosphate forming phosphate and hydroxybenzene.

• Then catalyzed by Potassium ferricyanide($K_3Fe(CN)_6$), hydroxybenzene combines with 4-aminophenazone (4-AAP) to form a red complex.

\[
\begin{align*}
\text{phenyl PO}_3\text{Na}_2 + \text{H}_2\text{O} & \xrightarrow{\text{ALP} \quad \text{pH 10}} \text{phenyl OH} + \text{Na}_2\text{HPO}_4 \\
\text{K}_3\text{Fe(CN)}_6 & \rightarrow \text{red quinone complex}
\end{align*}
\]
Sample: serum

- Substrate solution: Disodium phenyl phosphate
- Standard solution: hydroxybenzene (0.05mg/ml)
- Buffer carbonate: pH=10.0, 4-aminophenazone
- Color reagent: Potassium ferricyanide ($K_3Fe(CN)_6$), Boric acid

Function: stop the reaction, reveal color

dH$_2$O
<table>
<thead>
<tr>
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<th>T</th>
<th>S</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>Serum (ml)</td>
<td>0.1</td>
<td>—</td>
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<tr>
<td>Standard solution (ml)</td>
<td>—</td>
<td>0.1</td>
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<tr>
<td>dH₂O (ml)</td>
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<td>0.1</td>
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<tr>
<td>Buffer carbonate (ml)</td>
<td>1.0</td>
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<tr>
<td><strong>Mix, put them and substrate solution at 37°C for 5 min</strong></td>
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<tr>
<td>Substrate solution (ml)</td>
<td>1.0</td>
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<tr>
<td><strong>Mix, at 37°C for 15 min (exactly)</strong></td>
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<tr>
<td>Color reagent (ml)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Serum (ml)</td>
<td>—</td>
<td>—</td>
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<td>0.1</td>
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- **Mix well, measure the absorbance of each tube setting zero with dH₂O, λ=510nm.**
• ALP activity $=\frac{(A_T-A_C)}{(A_S-A_B)} \times 0.05 \times 100$ (kings unit)

• The definition of one kings unit:
  - the activity of the enzyme in 100ml serum that catalyzes substrate for 15 min at $37^\circ$C producing 1mg hydroxybenzene.

• EXPECTED NORMAL VALUE

  Children: 5 ~ 28 kings unit

  Adults: 3 ~ 13 kings unit
• An enzyme test is a blood test or urine test that measures levels of certain enzymes to assess how well the body’s systems are functioning and whether there has been any tissue damage (why?)
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• In healthy individuals, the levels of these enzymes are fairly constant.
• Common enzymes used for clinical diagnosis include:
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  • Alanine aminotransferase (ALT or GPT)
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  • Amylase
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  • Alanine aminotransferase (ALT or GPT)
    • Alkaline phosphatase (ALP)
  • Amylase
  • Aspartate aminotransferase (AST or GOT)
  • Creatine kinase (CK)