The procedure is intended for the qualitative and/or semi-quantitative determination of serum alkaline phosphatase isoenzymes using specimen pretreatment with neuraminidase and agarose electrophoresis on the SPIFE Alkaline Phosphatase Isoenzyme system.

### Summary

**Alkaline Phosphatase (ALP)** (EC 3.1.3.1.) is an enzyme which catalyzes the hydrolysis of phosphate esters at an alkaline pH. The greatest concentration of this enzyme is found in the liver, intestine, and bone. However, practically every body tissue contains at least a small amount of ALP. ALP differs significantly in chemical and physiologic properties and, by taking advantage of these differences, the individual isoenzymes can be identified. Using the SPIFE Alkaline Phosphatase Isoenzyme procedure, the isoenzyme migrations differ from those seen in conventional isoenzyme electrophoretic methods.

**Bone ALP isoenzyme** is an isoenzyme of ALP contained in bone tissue. A progressive rise begins about 32 to 34 weeks gestation, with relatively inconsistent values prior to this. The isoenzyme is heat stable and readily identifiable in the lab. If the heat stable isoenzyme level is low, the prognosis for the fetus is ominous. Little information is gained if the value falls within the normal range.

### Performance Characteristics

- **Precision**
  - Between Run studies were done using a patient sample run in replicate on one gel. N = 40
  - Within Run a specimen was run in replicate on five (5) gels. N = 200

- **Correlation:**
  - Between Run studies were done using 114 normal and abnormal patient samples and controls comparing the REP Alkaline Phosphatase method and the SPIFE Alkaline Phosphatase method using the SPIFE 3000. N = 114
  - Within Run studies were done using a patient sample run in replicate on one gel. N = 100

### Storage and Stability

- The dry powder should be stored at 2 to 8°C and is stable until the expiration date on the vial. It is preferable to refrigerate the blood specimen hours after a fatty meal.

### Patient Preparation:

- It is preferable to refrigerate the blood specimen.

### Ingredients:

- **Magnesium Sulfate**

### Analytical Information

- **Signs of Deterioration:**
  - The gels should be stored at room temperature (15 to 30°C) in the protective packaging and are stable until the expiration date indicated on the package.
  - The product is ready for use as packaged.
  - Storage and Stability: Store the Destain at 2 to 8°C and is stable until the expiration date on the package.

### Instructions for Use

- The product is used for in-vitro diagnostic use only. DO NOT INGEST.

### Troubleshooting

- The destain contains 3% (w/v) citric acid. DO NOT INJECT.

### References

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Gels should be scanned and/or interpreted

III. Sample Preparation

1. Dispense approximately 2 mL of REP Prep onto the left side of the electrophoresis chamber.
2. Place the left edge of the gel over the REP Prep and fold the gel rearward and over the left side of the chamber. Gently press the gel down, starting from the left side and ending on the right side. If using the cover plate, tilt the cover plate right over the right edge of the gel under the plate. If using left-freeze solution, place over the left edge of the gel over the edges of the plastic gel backing, especially next to the electrode pads, to remove excess REP Prep. Make Sure no air bubbles remain under the gel.
3. Thoroughly wash the slides with deionized water before and after each use. Wipe the carbon electrodes with a lint-free tissue.
4. Position the electrophoresis chamber on the gel block area.
5. Place the T-PRESS TEST SELECT/CONTINUE button on the electrophoresis and Stainer sides of the instrument unit ALKALINE PHOSPHATASE.

V. Sample Application/Electrophoresis

1. Place the Sample Tray with samples on the SPIFE 3000. Align the holes of the tray with the pins of the instrument. The tray will slide into the instrument unit.
2. With the end of the tray facing the left pin of the chamber lid, insert the tray into the instrument through the left hole of the chamber. The tray will stop before the right hole of the chamber.
3. Close the chamber lid.
4. With the end of the tray facing the left pin of the chamber, turn the T-PRESS button to start the test.
5. Open the chamber lid and remove the chamber cover.
6. Close the chamber lid.

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Quality Control: The GEL Alkaline Phosphatase Isoenzyme Control (Cat. No. 5821) is available and should be used with the gel to ensure that the gel is of proper quality before use. The test results from the GEL Alkaline Phosphatase Isoenzyme Control is quantitated, an approximate value for the patient sample can be derived by comparison to the control. The results can be reported as greater than or less than the control values.

Stability of End Product: Gels should be scanned and/or interpreted within 2 hours of gel being loaded.
STEP-BY-STEP

NOTE: If a SPIFE procedure requires a stain that has run prior to using the ALP gels, the stain should be cleaned/washed before using the gel.

1. If testing 21 to 40 samples, remove two 1115 disposable Applicator Blade Assemblies.
2. Place a reconstituted vial of reagent in the center hole of the REP Prep. If cleaning is required, the "Wash" prompt will appear, followed by "Plate out. Holder in" prompts. Press "Continue" to begin the stainer wash cycle. The stainer will wash the previous test 10 minutes is acceptable. *A Dry 1 time of 10 minutes is recommended, but a range of 10 to 20 minutes is acceptable.** An electrophoresis time of 27 minutes is acceptable, with a range of 20 to 30 minutes is acceptable.

A. SPIFE 3000

1. Place the Sample Tray with samples on the SPIFE 3000. Align the holes in the tray with the pins on the instrument. The SPIFE 2000 will apply the samples and beep when completed.
2. Place a Chamber Cover over the right pin on the holder and the obround hole over the right pin on the outside of magnets. Make sure no tissue to wipe around the edges of the plastic gel backing, especially at the gel block area.
3. Place an empty Gel Holder in the stainer unit. If cleaning is required, the system will know this.
4. Place a reconstituted vial of reagent in the center hole of the REP Prep. If cleaning is required, the "Wash" prompt will appear, followed by "Plate out. Holder in" prompts. Press "Continue" to begin the stainer wash cycle. The stainer will wash the previous test 10 minutes is acceptable. *A Dry 1 time of 10 minutes is recommended, but a range of 10 to 20 minutes is acceptable.** An electrophoresis time of 27 minutes is acceptable, with a range of 20 to 30 minutes is acceptable.

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It is preferable to refrigerate the blood specimen. A normal isoenzyme pattern should separate bone and intestinal isoenzymes clearly. Each gel contains agarose in a tris-barbital-sodium barbital buffer. An abnormally high bone isoenzyme level may also be indicative of bone cancer, osteosarcoma or coeliac sprue. A decreased bone ALP in children may be attributed to cretinism or to hypophosphatasia. An intestinal isoenzyme band may be seen in patients with disease states such as celiac disease, inflammatory bowel disease, coeliac sprue, malabsorption, and sprue. It is elevated in these patients postprandially and after a fatty meal. Additionally, the intestinal band is found in disease states such as celiac disease, inflammatory bowel disease, coeliac sprue, malabsorption, and sprue.

**Storage and Stability:** The dry powder should be stored at 2 to 8°C and is stable until the expiration date on the bottle. The reconstituted reagents and chromogen should be used within 30 minutes. Storage at temperatures below 2°C for more than 24 hours may result in color differences.

**Bone Alkaline Phosphatase Diluent:**
- 2-Amino-2-Methyl-1-Propanol 2.0 M
- 5-Bromo-4-Chloro-3-Indolyl Phosphate-β-Toluene sulfonic acid 1.7 M
- Magnesium Sulfate 0.05 M
- Sodium Azide 0.05 M

**Warning:** For in-vitro diagnostic use only. Do not inject.

**Preparation for Use:**
- The diluent is ready for use as packaged.
- The reagents are stable at 2 to 8°C and are stable until the expiration date on the bottle.
- Significant coagulation of the specimen should not be detected if it becomes milky white or shows signs of contamination.

**SPIFE Alkaline Phosphatase (ALP) Test Kit**

- **Materials Provided:**
  - Alkaline Phosphatase Diluent
  - Citric Acid Destain
  - Neuraminidase from Clostridioides difficile

- **Preparation for Use:**
  - Store the test kit at 2 to 8°C and is stable until the expiration date on the package.

**Warning:** For in-vitro diagnostic use only. Do not inject.

**Procedure:**
- The product is ready for use as packaged.
- Store the test kit at 2 to 8°C and is stable until the expiration date on the package.

**Compatibility:**
- The test kit is compatible with all SPIFE 2000/3000 instruments.

**Warning:** For in-vitro diagnostic use only. Do not inject.

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