PHOSPHATASES, ALKALINE, ACID, PROSTATIC ACID

(Procedure No. 104)

Revised: 2001-11

INTENDED USE

Sigma Diagnostics Alkaline, Acid and Prostatic Acid Phosphatase kits are intended for use in the quantitative, colorimetric determination of alkaline, acid and prostatic acid phosphatases, respectively, in serum at 400–420 nm.

BACKGROUND AND PRINCIPLE OF TEST

In 1930, Kay1 demonstrated the presence of alkaline phosphatase in blood using 8-glyceroephosphoryl as substrate. Subsequently, others using this and different substrates2–4 improved the technique and broadened the test to measure both acid phosphatase and prostatic acid phosphatase in serum.

Early procedures relied on measurement of liberated phosphate from the substrate in the presence of pre-existing phosphate in serum. Thus, high blanks were frequently encountered that reduced the reliability of the phosphatase determination. To overcome this shortcoming, King and Armstrong5 introduced use of phenylphosphate as substrate for phosphatase and prostatic acid phosphatase in serum.

The Sigma procedure for determination of alkaline phosphatase utilizes 2-amino-2-methyl-1-propanol (AMP) buffer and involves only a 15-minute incubation. Serum acid phosphatase is derived from a number of tissues, principally the prostate, liver and spleen, as well as erythrocytes, leukocytes and platelets. A number of techniques have been devised for specifically distinguishing prostatic acid phosphatase because of its importance in the diagnosis of carcinoma of the prostate with metastasis. Since tartrate abolishes about 95% of prostatic acid phosphatase activity, it serves as a useful basis for assessing the level of this enzyme in serum.

The Sigma procedures for acid and alkaline phosphatase depend upon the hydrolysis of p-nitrophenyl phosphate by the enzymes, yielding p-nitrophenol and inorganic phosphate. When made alkaline, p-nitrophenol is converted to a yellow complex readily measured at 400–420 nm. The intensity of color formed is proportional to phosphatase activity.

REAGENTS

SIGMA 104® PHOSPHATASE SUBSTRATE, p-Nitrophenyl phosphate, disodium

40 mg Capsule, Catalog No. 104-40

100 mg Capsule, Catalog No. 104-100

Bulk package, Catalog No. 104-0

CITRATE BUFFER SOLUTION, Catalog No. 104-4

Citrate, 90 mmol/l, and chloride, 10 mmol/l, pH 4.8 at 25°C. Chloriform added as preservative.

p-NITROPHENOL STANDARD SOLUTION, Catalog No. 104-1

p-Nitrophenol, 10 mmol/l

TARTRATE ACID BUFFER SOLUTION, Catalog No. 104-12

L (+) Tartaric acid, 40 mmol/l, in citrate buffer, 90 mmol/l, pH 4.8 at 25°C. Chloriform added as preservative.

221 ALKALINE BUFFER SOLUTION, Catalog No. 221

2-Amino-2-methyl-1-propanol, 1.5 mol/l, pH 10.3 at 25°C.

PRECAUTIONS:

Acid and Alkaline Phosphatase reagents are for “In Vitro Diagnostic Use”. Normal precautions exercised in handling laboratory reagents should be followed. Wear suitable protective clothing, gloves and eye/face protection. Dispose of waste observing all precautions exercised in handling laboratory reagents should be followed. Wear suitable protective clothing, gloves and eye/face protection. Refer to Material Safety Data Sheets for any updated risk, hazard or safety information.

PREPARATIONS:

Stock Substrate Solution: Remove Sigma 104 Phosphatase Substrate from freezer and allow to warm to room temperature before opening package to avoid moisture pick-up. The Stock Substrate Solution is prepared according to the following table.

SUBSTRATE

WATER

40 mg Capsule, Catalog No. 104-40

100 mg Capsule, Catalog No. 104-100

Diluted p-Nitrophenol Standard Solution is prepared by pipetting 0.5 ml p-Nitrophenol Standard Solution into a 100 ml volumetric flask. Dilute to 100 ml with 0.02 N sodium hydroxide solution. Mix thoroughly.

Refer to “Reagents Required But Not Provided” section for preparation of acid and base solutions that may be needed.

STORAGE AND STABILITY:

Store Sigma 104 Phosphatase Substrate in the freezer (below 0°C). Substrate should be off-white to yellowish and is suitable for use as long as reagent blank can be brought to zero absorbance in the instrument.

Stock Substrate Solution should be dispensed in 0.5 ml aliquots into incubation tubes, stopped and frozen in an upright position. Stable six weeks in the freezer (below 0°C).

Store Citrate Buffer Solution, Tartrate Acid Buffer Solution and 221 Alkaline Buffer Solution in refrigerator (2–8°C). Buffers are suitable for use in the absence of microbial growth.

Store p-Nitrophenol Standard Solution in refrigerator (2–8°C). Protect from light. Reagent is stable until expiration date.


REAGENTS REQUIRED BUT NOT PROVIDED

NOTE: Use ACS grade chemicals throughout for reagent preparation.

HYDROCHLORIC ACID, Concentrated (For Serum Alkaline Phosphatase Procedure)

SODIUM HYDROXIDE SOLUTION, 0.1 N (For Serum Total Acid and Prostatic Acid Phosphatase Procedures) Prepare by dissolving 4.0 g sodium hydroxide in 1000 ml deionized water.

SODIUM HYDROXIDE SOLUTION, 0.05 N

Prepare by dissolving 2.0 g sodium hydroxide in 1000 ml deionized water.

SODIUM HYDROXIDE SOLUTION, 0.02 N

Prepare by dissolving 0.8 g sodium hydroxide in 1000 ml deionized water.

OPTIONAL REAGENTS

Reagents for preservation of total acid and prostatic acid phosphatase activity:

ACP STABILIZER, Catalog No. A 2170

Acetate buffer, 5 mol/l. Add 0.02 ml to 1 ml serum.

PROSTATIC ACID PHOSPHATASE STABILIZER TABLETS, Catalog No. 104-9

Tablets contain citric acid, 4 mg, and excipient. Add one tablet to 1 ml serum.

NOTE: Suitability for use can be verified by dissolving 1 tablet per ml serum and measuring pH, which should be between 5 and 6.

SPECIMEN COLLECTION AND STORAGE

It is recommended that specimen collection be carried out in accordance with NCCLS document M29-T2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.

SERUM:

Blood should be collected without stasis or hemolysis. Ruptured red cells release acid phosphatase which is present in higher concentrations within the cell than in serum. Several authors14–16 report that prostastic massage may elevate serum total and prostatic acid phosphatase. It is recommended that 24 to 48 hours elapse after treatment before obtaining serum for acid phosphatase determination.

STABILITY OF HUMAN SERUM PHOSPHATASE:

Alkaline phosphatase in serum is stable for at least 8 days at room temperature17 and longer when frozen.18 By contrast, acid phosphatase rapidly loses activity at room temperature. Several sera, for example, which initially exhibited 20 units of total acid phosphatase activity, yielded only 1.9 units of activity after storage for 2 hours at 37°C.19 At 25°C, the drop was less but still appreciable. Therefore, 50–90% of serum acid phosphatase activity can disappear within a few hours on a warm day. King and Jegatheesan20 found acid phosphatase stable at 0°C up to 14 days, provided the serum was refrigerated immediately after separation from clot. It is suggested that promptly after collection, blood be placed in a container of cracked ice or refrigerated. Within 1 hour, centrifuged blood, separate serum and refrigerate. If ACP Stabilizer (0.02 ml) or a Stabilizer Tablet, Catalog No. 104-9, is added to 1 ml serum, inactivation of prostatic acid phosphatase is minimized for several days even at 37°C. This preservation is useful when serum is to be mailed.

If alkaline phosphatase is to be determined as well, divide the serum before treatment for acid phosphatase determination.

If ACP Stabilizer (0.02 ml) or a Stabilizer Tablet, Catalog No. 104-9, is added to 1 ml serum, inactivation of prostatic acid phosphatase is minimized for several days even at 37°C. This preservation is useful when serum is to be mailed.

PLASMA PHOSPHATASE:

Heparinized plasma may be used for alkaline phosphatase. Fluoride and anti-coagulants such as citrate, oxalate or EDTA should be avoided as these agents inhibit alkaline phosphatase activity. Plasma obtained using ACD (acid-citrate-dextrose) may be used for acid phosphatase determination.23 Do not use fluoride, heparin, EDTA or oxalate.24–26

STORAGE AND STABILITY:

Store Sigma 104 Phosphatase Substrate in the freezer (below 0°C). Substrate should be off-white to yellowish and is suitable for use as long as reagent blank can be brought to zero absorbance in the instrument.

Stock Substrate Solution should be dispensed in 0.5 ml aliquots into incubation tubes, stopped and frozen in an upright position. Stable six weeks in the freezer (below 0°C).

Store Citrate Buffer Solution, Tartrate Acid Buffer Solution and 221 Alkaline Buffer Solution in refrigerator (2–8°C). Buffers are suitable for use in the absence of microbial growth.

Store p-Nitrophenol Standard Solution in refrigerator (2–8°C). Protect from light. Reagent is stable until expiration date.

Tissue Phosphatase:
Prepare extract and proceed as for serum. Adjust volume assayed to the activity contained per ml.

Leukocyte Alkaline Phosphatase:
In 1970, DeChatelet and Cooper described the determination of leukocyte alkaline phosphatase using Sigma 104 Phosphatase Substrate. It was demonstrated that AMP buffer yielded adequate results.

INTERFERING SUBSTANCES:
1. According to Sigma technique, serum alkaline phosphatase reaction mixtures are decolorized with acid after photometric measurement of the yellow alkaline p-nitrophenol color. Any residual color normally accounts for other serum chromogens and is subtracted to obtain correct alkaline phosphatase activity. Nevertheless, bilirubin and hemoglobin, if present in high concentration, can introduce error since these pigments have slightly greater absorptions in alkali than in acid. From a practical standpoint, this error is small enough to ignore. If desired, a correction can be made by preparing a SERUM BLANK containing 0.10 ml serum and 11.0 ml of 0.02 N NaOH.
2. A SERUM BLANK is routinely employed in the procedures for total acid and prostatic acid phosphatase to correct for color contributed by serum.
3. Specimens visibly hemolyzed are not suitable for test purposes as red blood cells are rich in acid phosphatase.

Instrument and Materials Required

INSTRUMENT:
Centrifuge

MATERIALS:
Conventional or automatic pipets may be used. Automatic diluters which have demonstrated acceptable reliability may also be employed. If conventional pipets are used, the following sizes will be needed: 0.1, 0.2, 0.5, 1, 2 and 10 ml serologic.

Water bath at 37°C

SERUM ALKALINE PHOSPHATASE:

PROCEDURES
1. Pipet into each of 2 tubes: 0.5 ml 221 Alkaline Buffer Solution, Catalog No. 221 0.5 ml Stock Substrate Solution Place both tubes in a 37°C water bath to equilibrate.
2. Pipet 0.1 ml water into tube labeled BLANK.
NOTE: Only one BLANK is needed for each series of tests.
3. Pipet 0.1 ml serum into tube labeled TEST. Record exact time, mix gently and replace in water bath promptly.
4. After exactly 15 minutes, add 10.0 ml 0.05 N NaOH to each tube and mix by inversion.
NOTE: Alkaline phosphatase activity.
5. Read absorbance of TEST vs BLANK as reference at 400–420 nm. Determine alkaline phosphatase units corresponding to this reading from calibration curve.
6. Add 4 drops (0.2 ml) concentrated HCl to each tube and mix.
NOTE: Acid removes color due to p-nitrophenol, leaving absorbance due to serum.
7. Again read absorbance of TEST using BLANK as reference. Determine alkaline phosphatase units corresponding to this reading from calibration curve.
8. Subtract the alkaline phosphatase activity of Step 7 from alkaline phosphatase activity of Step 5, yielding corrected alkaline phosphatase activity of serum.
NOTE: With values greater than 10 Sigma Units/ml, repeat assay using a 5-minute incubation and multiply results by 3. If value is still too high, use less serum and multiply by appropriate factor.

SERUM TOTAL ACID PHOSPHATASE:

CAUTION: Refrigerate blood immediately after drawing. Centrifuge within 1 hour. Store unhemolyzed serum in refrigerator (2–8°C). If serum cannot be refrigerated, add Prostatic Acid Phosphatase Stabilizer Tablet, Catalog No. 104-9 (1 tablet/ml) or add ACP Stabilizer (0.02 ml/m1 serum) to preserve enzyme activity.

1. Remove two Stock Substrate Solution tubes each containing 0.5 ml aliquots from freezer and place in 37°C water bath. Pipet 0.5 ml Citrate Buffer, Catalog No. 104-4, into each tube, mix and bring to water bath temperature.
2. Pipet 0.2 ml water into tube labeled REAGENT BLANK.
3. Pipet 0.2 ml serum into tube labeled TEST. Record exact time, mix gently and return to water bath for 30 minutes.
4. Prepare SERUM BLANK by mixing 6.0 ml 0.1 N NaOH with 0.2 ml serum.
5. Read absorbance of SERUM BLANK vs water as reference at 400–420 nm. Obtain units of acid phosphatase corresponding to this reading from calibration curve.
6. Exactly 30 minutes after adding serum to substrate (Step 3), pipet 5 ml 0.1 N NaOH into REAGENT BLANK and TEST. Stopper and mix by inversion.
NOTE: Alkaline phosphatase activity.
7. Read absorbance of TEST using REAGENT BLANK as reference. Determine acid phosphatase units corresponding to this reading from calibration curve.
8. Subtract the acid phosphatase activity of Step 5 from the acid phosphatase activity in Step 7, yielding corrected total acid phosphatase activity of serum.
NOTE: With values greater than 2.8 Sigma Units/ml, repeat using a 15-minute incubation and multiply results by 2. If results are still too high, use less serum and multiply by appropriate factor.
QUALITY CONTROL:

The reliability of test results may be monitored by routine use of frozen aliquots of human serum pools or commercially available serum preparations of known alkaline and acid phosphatase activity. Sigma offers two serum enzyme controls containing several enzymes including alkaline and total acid phosphatase. Sigma Enzyme Control 2-N, Catalog No. S 1005, contains alkaline and total acid phosphatase at normal levels. Sigma Enzyme Control 2-E, Catalog No. S 1005, contains enzymes at elevated levels. Controls do NOT contain prostatic acid phosphatase. Values are stated in Sigma Units as determined by the described procedure.

RESULTS

Results are derived as described in “Procedures” section and expressed in Sigma Units. A Sigma Unit is defined as that amount of enzyme activity which will liberate 1 μmol of p-nitrophenol per hour under the test conditions described by Bessey et al.12

EXAMPLE: Serum Alkaline Phosphatase
Absorbance of TEST (Step 5) = 0.250
Absorbance of TEST after HCI (Step 7) = 0.010

By referring to Figure 1, absorbance of TEST before and after HCI addition correspond to alkaline phosphatase activity of 2.1 and 0.1 Sigma Units/ml, respectively. By subtraction (2.1 – 0.1), the corrected serum alkaline phosphatase activity equals 2.0 Sigma Units/ml.

EXAMPLE: Serum Total Acid Phosphatase
Absorbance of TEST (Step 7) = 0.250
Absorbance of SERUM BLANK (Step 5) = 0.025

By referring to Figure 2, absorbances of TEST and SERUM BLANK correspond to total acid phosphatase activities of 0.62 and 0.05 Sigma Units/ml, respectively. By subtraction (0.62 – 0.05), the corrected serum total acid phosphatase is 0.57 Sigma Units/ml.

EXAMPLE: Serum Prostatic Acid Phosphatase
Absorbance of Tube 1, with tartrate buffer (Step 6) = 0.200
Absorbance of Tube 2, with citrate buffer (Step 6) = 0.250

By referring to Figure 2, absorbances of Tube 1 and Tube 2 correspond to serum acid phosphatase activities of 0.49 and 0.62 Sigma Units/ml, respectively. By subtraction (0.49 – 0.48), the corrected serum prostatic acid phosphatase activity is 0.14 Sigma Units/ml.

INTERNATIONAL UNITS:

A Sigma Unit of phosphatase activity is defined as that amount of enzyme activity that will liberate 1 μmol of p-nitrophenol per hour under the test conditions described by Bessey et al.12

Please note that the alkaline phosphatase procedure formerly employed glycine as buffer. The use of AMP buffer results in essentially twice the activity as that obtained with tartrate buffer. The use of AMP buffer results in essentially twice the activity as that obtained with tartrate buffer.

EXPECTED VALUES

<table>
<thead>
<tr>
<th>NORMAL SERUM ALKALINE PHOSPHATASE①①②③</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults: 0.8–3.0 Sigma Units/ml (13–50 U/l)</td>
</tr>
<tr>
<td>Children: 2.6–6.7 Sigma Units/ml (47–112 U/l)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NORMAL TOTAL SERUM ACID PHOSPHATASE⑤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males: 0.13–0.63 Sigma Units/ml (2–11 U/l)</td>
</tr>
<tr>
<td>Females: 0.01–0.56 Sigma Units/ml (0.2–10 U/l)</td>
</tr>
</tbody>
</table>

SERUM PROSTATIC ACID PHOSPHATASE**

Normal: 0.01–0.15 Sigma Units/ml (0.2–2.5 U/l)
Borderline: 0.15–0.20 Sigma Units/ml (2.5–3.0 U/l)

Normal ranges stated in this procedure were taken from the literature. The cited methods used to obtain these values are similar to those described in this procedure and results should be applicable. Copeland26 suggests that each laboratory determine its own normal range. Attention should be given to the fact that certain measurements are influenced in clinically healthy individuals by diet, sex, age, diurnal variation, physical activity, menstrual cycle, pregnancy and environmental factors.26 Administration of certain drugs and medications has been shown to influence body levels of phosphatase activity. A comprehensive review has been prepared by Young et al., and should be consulted for further information.

SERUM PHOSPHATASES IN DISEASES:

Values have been compiled from several sources and may serve as a guide for interpretation of elevations encountered in various disorders.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Alkaline Phosphatase (Sigma Units/ml)</th>
<th>Total Acid Phosphatase (Sigma Units/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic cirrhosis</td>
<td>2.5–10.0</td>
<td>&gt; 20 Occasional Elevated</td>
</tr>
<tr>
<td>Hyperparathyroidism</td>
<td>10.0–14.0</td>
<td></td>
</tr>
<tr>
<td>Jaundice</td>
<td>0.8–2.5</td>
<td></td>
</tr>
<tr>
<td>Hemolytic</td>
<td>2.5–5.0</td>
<td></td>
</tr>
<tr>
<td>Nonobstructive</td>
<td>2.5–45.0</td>
<td></td>
</tr>
<tr>
<td>Obstructive</td>
<td>2.5–45.0</td>
<td></td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>Moderately Elevated</td>
<td></td>
</tr>
<tr>
<td>Generalized</td>
<td>2.7–5.0</td>
<td></td>
</tr>
<tr>
<td>Senile</td>
<td>1.5–3.0</td>
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</tr>
<tr>
<td>Osteosclerosis</td>
<td>8.0–12.0</td>
<td></td>
</tr>
<tr>
<td>Paget's Disease</td>
<td>Misc: Pseudobrusen Diseases</td>
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</tr>
<tr>
<td>Localized</td>
<td>2.7–10.0</td>
<td></td>
</tr>
<tr>
<td>Polystatic</td>
<td>10.0–75.0</td>
<td></td>
</tr>
<tr>
<td>Rickets</td>
<td>Prostate Paipation (up to 48 h)</td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>15.0–90.0</td>
<td></td>
</tr>
<tr>
<td>Healed</td>
<td>3.0–6.0</td>
<td></td>
</tr>
</tbody>
</table>

Alkaline phosphatase levels tend to parallel osteogenic activity. Therefore, serum alkaline phosphatase activity is higher in children than in adults. A rise of 2–3 times normal is observed in the third trimester of pregnancy. Decreased serum alkaline phosphatase values are infrequently encountered. Low levels are sometimes found in cases of hypothyroidism, scurvy, celiac disease, severe chronic nephritis and pernicious anemia.

SERUM PROSTATIC ACID PHOSPHATASE IN DISEASE:

Many early reports showed poor correlation between serum acid phosphatase activity and cancer of the prostate. However, the extreme instability of acid phosphatase was not recognized. Thus, normal values were probably obtained on sera originally having high acid phosphatase levels. Also, most of these reports failed to differentiate between prostatic and total acid phosphatase.

Prostatic acid phosphatase is sometimes markedly elevated even when total acid phosphatase is normal. Bonner et al., for example, reported that among 39 untreated patients with prostatic carcinoma, 34 had elevated prostatic acid phosphatase values while only 12 had increased total acid phosphatase. The remaining patients had normal total and prostatic acid phosphatase levels.

Ozor et al., reported on the use of tartrate to inhibit prostatic acid phosphatase as a means of differentiating cases of prostate cancer from other diseases associated with elevated serum total acid phosphatase. These workers found in a group of 15 prepubertal patients, 14 with elevated total acid phosphatase and 5 with increased prostatic acid phosphatase activity. In these cases, the high prostatic acid phosphatase was not indicative of carcinoma of the prostate.

Jacobsson reported that 123 out of 125 hospitalized men over age 60 had normal tartrate-inhibited acid phosphatase values when determined by the Sigma procedure, but at pH 5.5. The remaining two patients exhibited increased values and "may well have had cancer of the prostate".

PERFORMANCE CHARACTERISTICS

REPRODUCIBILITY STUDIES:

The coefficient of variation (CV) for serum alkaline phosphatase at normal levels is 2% with a standard deviation of 0.04 Sigma Units/ml. At levels below 1.0 Sigma Units/ml, the CV is 6% and in the elevated range the CV drops to 1%. For total acid phosphatase, the reproducibility studies follow the same pattern, normal levels having a CV of 2% with a standard deviation of 0.04 Sigma Units/ml, the CV is 6% and in the elevated range the CV drops to 1%.

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Please note that the alkaline phosphatase procedure formerly employed glycine as buffer. The use of AMP buffer results in essentially twice the activity as that obtained when using glycine. To avoid confusion that might be caused among long established users of the original method if a new unit were adopted, results are expressed in terms of the same Sigma Unit.

Phosphatase activity in Sigma Units/ml may be converted to International Units (U/l) by multiplying by 16.7. For example, serum alkaline phosphatase activity of 3 Sigma Units/ml equals 50 U/l.

![Figure 2: Typical Acid Phosphatase Calibration Curve](image-url)
CORRELATION STUDIES:

Ten samples were analyzed for alkaline phosphatase by Sigma and SMA 12/60 procedures based on the Bessey-Lowry-Brock principle. Comparable results were obtained by both methods in the normal and elevated ranges.

Sigma 104® is a registered trademark of Sigma-Aldrich Co., St. Louis, MO

Sigma Diagnostics, Inc., warrants that its products conform to the information contained in this and other Sigma publications. Purchaser must determine the suitability of the product for its particular use. See reverse side of invoice or packing slip for additional terms and conditions of sale.

REFERENCES

19. Data obtained by Sigma Diagnostics

ORDERING INFORMATION

ALKALINE PHOSPHATASE KITS

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>104-LS</th>
<th>104-LI</th>
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<tbody>
<tr>
<td>Quantity</td>
<td>40</td>
<td>200</td>
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</tbody>
</table>

Contents - Catalog Numbers

- SIGMA 104® Phosphatase Substrate, 104-40
  - 2 capsules
- SIGMA 104® Phosphatase Substrate, 104-100
  - 4 capsules

221 Alkaline Buffer Solution (AMP), 221
- 25 ml
- 100 ml
- p-Nitrophenol Standard Solution, 104-1
  - 7 ml
  - 7 ml

ACID PHOSPHATASE, TOTAL

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>104-AS</th>
<th>104-AL</th>
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<tbody>
<tr>
<td>Maximum Assays</td>
<td>40</td>
<td>200</td>
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Contents - Catalog Numbers

- SIGMA 104® Phosphatase Substrate, 104-40
  - 2 capsules
- SIGMA 104® Phosphatase Substrate, 104-100
  - 4 capsules

Citrine Buffer Solution, 104-4
- 25 ml
- 100 ml
- p-Nitrophenol Standard Solution, 104-1
  - 7 ml
  - 7 ml

ACID PHOSPHATASE, TOTAL AND PROSTATIC

<table>
<thead>
<tr>
<th>Catalog No.</th>
<th>104-ATL</th>
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<tbody>
<tr>
<td>Maximum Assays</td>
<td>100</td>
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</tbody>
</table>

Contents - Catalog Numbers

- SIGMA 104® Phosphatase Substrate, 104-100
  - 4 capsules
- Citrine Buffer Solution, 104-4
  - 100 ml
- p-Nitrophenol Standard Solution, 104-1
  - 7 ml

NOTE: Kits do not contain sodium hydroxide (Catalog No. 506-8), hydrochloric acid or acetic acid which are also needed.

INDIVIDUAL REAGENTS

<table>
<thead>
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<th>Catalog No.</th>
<th>Item</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>104-1</td>
<td>p-NITROPHENOL STANDARD SOLUTION</td>
<td>7 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25 ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100 ml</td>
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</table>

104-4 CITRINE BUFFER SOLUTION
- 25 ml
- 100 ml
- 500 ml

221 221 ALKALINE BUFFER SOLUTION

<table>
<thead>
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<td>104-40</td>
<td>SIGMA 104® PHOSPHATASE SUBSTRATE</td>
<td>10 capsules</td>
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<tr>
<td></td>
<td>Preweighed 40 mg capsules</td>
<td>25 capsules</td>
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<tr>
<td>104-100</td>
<td>SIGMA 104® PHOSPHATASE SUBSTRATE</td>
<td>10 capsules</td>
</tr>
<tr>
<td></td>
<td>Preweighed 100 mg capsules</td>
<td>25 capsules</td>
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<tr>
<td>104-0</td>
<td>SIGMA 104® PHOSPHATASE SUBSTRATE</td>
<td>250 mg</td>
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<td>500 mg</td>
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OPTIONAL REAGENTS

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<td>A 2170</td>
<td>ACP STABILIZER</td>
<td>10 ml</td>
</tr>
<tr>
<td>104-9</td>
<td>PROSTATIC ACID PHOSPHATASE STABILIZER TABLETS</td>
<td>100 tablets</td>
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<tr>
<td>506-8</td>
<td>SODIUM HYDROXIDE, Anhydrous</td>
<td>16 g vial</td>
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</table>

SIGMA ENZYME CONTROLS

| S 0005 | 2-E, Elevated | 10x3 ml |
| S 0005 | 2-N, Normal | 10x3 ml |

Procedure No. 104
Previous Revision: 1997-09
Revised: 2001-11

SIGMA DIAGNOSTICS, INC.
P.O. BOX 14508 ST. LOUIS, MO 63178 USA
Technical Service: 800-325-0250 or call collect 314-286-7880
To Order: 800-325-3010 or call collect 314-771-5750
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