The pepsinogen-1 test kit contains sufficient reagents for testing over 40 serum samples on microtiter wells.

Materials Supplied

1. Microwell Strips (pepsinogen-1 MAB coated) .............. 12 Strips
2. HRP-MAB conjugate (10x conc.) .............................. 1 x 2 mL
3. Enzyme conjugate diluent ......................................... 1 x 20 mL
4. Serum sample diluent (10x conc.) ............................... 1 x 2 mL
5. Calibrator 1, 2, and 3 ...................................................... 3 x 2 mL
6. Negative Control ......................................................... 1 x 2 mL
7. Positive Control ......................................................... 1 x 2 mL
8. Substrate Solution A (TMB) ......................................... 1 x 8 mL
9. Substrate Solution B (H2O2) ......................................... 1 x 8 mL
10. Wash buffer (25x conc.) ............................................ 1 x 25 mL
11. Stop solution (2N H4SO4) ........................................... 1 x 6 mL

Store kit at 2-8°C.

IV. ADDITIONAL MATERIALS REQUIRED BUT NOT SUPPLIED

1. Distilled or deionized water
2. Absorbent paper towels to blot and dry the microtiter wells after washing, and parafilm/plastic wraps to cover during incubation.
3. Glass tubes for serum dilutions.
4. Micropipet with disposable tips to deliver 25 µL, 50 µL, and 100 µL volume.
5. A microtiter plate washer or a squeeze bottle for washing.
6. 100 µL pipets for substrate buffer and conjugate diluent delivery.
7. Nalgene, graduate cylinder.
8. Microtiter plate reader with 450 nm absorbance capability.

V. Warnings and Precautions

Potential Biohazardous Material

Since there is no test method that can offer complete assurance that test serum samples are free from HIV, Hepatitis B virus or other infectious agents, the serum samples should be handled as potentially infectious specimens, as recommended by the Center for Disease Control/National Institute of Health manual, “Bio-safety in microbiological and biomedical laboratories.”

Sodium Azide

Sodium azide that is present in reagents as preservative may react with lead, copper or brass to form explosive metal azide. To prevent the possible contamination of specimens, as recommended by the Center for Disease Control/National Institute of Health manual, “Bio-safety in microbiological and biomedical laboratories.”

Spline Regression

The reliability of the Pepsinogen-1 ELISA was assessed by conducting correlation experiments for reproducibility in detecting pepsinogen-1 antigen present in clinical serum samples.

Technical details for the correlation study were as follows: Three calibrators with different values (U/mL) are provided, covering low, medium and high values of pepsinogen-1 in human serum matrix. The exact value of each calibrator is indicated on the calibrator vial of each lot.

The pepsinogen-1 values are expressed in ng per mL of serum sample.

The reliability of the Pepsinogen-1 ELISA was assessed by conducting correlation experiments for reproducibility in detecting pepsinogen-1 antigen present in clinical serum samples.
**ICN-PEPSONIN-1 REPORT FORM (Sample)**

**Kit Lot #:** XXXX  **Expiration Date:** XXXX

**Assay:** XXXX  **Operator:** J. Doe

**Section A: Control Results**

<table>
<thead>
<tr>
<th>Controls</th>
<th>Mean O.D.</th>
<th>Peg-1 Value (µ/mL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0.363</td>
<td>22.0</td>
<td>—</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1.073</td>
<td>123</td>
<td>+</td>
</tr>
<tr>
<td>Calibrator #1</td>
<td>0.194</td>
<td>15.0</td>
<td>—</td>
</tr>
<tr>
<td>Calibrator #2</td>
<td>0.676</td>
<td>50.0</td>
<td>—</td>
</tr>
<tr>
<td>Calibrator #3</td>
<td>1.484</td>
<td>300.0</td>
<td>+</td>
</tr>
</tbody>
</table>

**NOTE:** Do not use these results for calculating values. This is only a sample.

**Section B: Patients' samples Results**

<table>
<thead>
<tr>
<th>Serum Sample</th>
<th>Ave. O.D.</th>
<th>Peg-1 Value (µ/mL)</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.551</td>
<td>52.5</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>0.429</td>
<td>38.2</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>0.943</td>
<td>159.3</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>1.039</td>
<td>215.6</td>
<td>+</td>
</tr>
</tbody>
</table>

**Date:** XXXXXX  **Reported by:** XXXX

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**REFERENCES:**


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**Contact:**

ICN Pharmaceuticals
Diagnostics Division
13 Mountain View Avenue
Orangeburg, NY 10962-1294

**Customer Service:** (800) 888-7008
**Fax:** (949) 260-1079
**www.icndiagnostics.com**

**07817681**
**QER NO.: 001-162**

7/01

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**Inter-Assay (between run) precision was determined by measuring O.D. values of 10 different runs with serum samples of Low and High Pepsinogen-1 values.**

**Specificity:**

The following closely related proteins (peptides) were tested for specificity against monoclonal antibody to human pepsinogen-1 and were compared with human pepsinogen-1.

- Porcine pepsinogen <1.0%
- Human pepsinogen-2 <0.1%
- Gastrin <0.1%
- Somatostatin <0.1%
- Bombesin <0.1%

As shown above, the cross reactivity of these proteins is very insignificant. The cross reactivity values may change slightly based on the protein preparations used in cross reactivity studies.

**Recovery studies** were performed using previously confirmed serum samples of known values of pepsinogen-1. Various concentrations of pepsinogen-1 were added to serum samples tested before. The results of the recovery studies are presented below.

<table>
<thead>
<tr>
<th>Serum Sample (U/mL)</th>
<th>Pepsinogen-1 Added (µ/mL)</th>
<th>Expected Value (U/mL)</th>
<th>Observed Value (U/mL)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>None</td>
<td>28</td>
<td>28</td>
<td>N/A</td>
</tr>
<tr>
<td>28</td>
<td>50</td>
<td>78</td>
<td>74</td>
<td>94.8</td>
</tr>
<tr>
<td>28</td>
<td>250</td>
<td>278</td>
<td>304</td>
<td>109.3</td>
</tr>
</tbody>
</table>

**The difference observed between expected and observed values show a percentage of recovery between 95 – 110% which is an acceptable variation range.**

**Sensitivity:**

The ICN-Pepsinogen-1 kit can detect pepsinogen-1 concentration of less than 1 ng in 100 µL of clinical samples.

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**XIII. SOURCE OF ERROR**

Poor test reproducibility may result due to:

- a. Inconsistent delivery of reagents
- b. Improper storage of reagents
- c. Improper reconstitution of reagents
- d. Incomplete washing of microwells
- e. Substrate reagent either old or exposed to light
- f. Unstable / defective spectrophotometer (ELISA reader)
- g. Error in following the assay procedure

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**Number of determinations**

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

**Mean O.D. value**

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.433</td>
<td>0.903</td>
</tr>
</tbody>
</table>

**S.D.**

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.031</td>
<td>0.062</td>
</tr>
</tbody>
</table>

**C.V.**

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.8%</td>
<td>8.3%</td>
</tr>
</tbody>
</table>