Type IV Collagen ELISA (Serum)

For the quantitative determination of collagen IV in human serum.

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 69-C4SHU-E01
Size: 96 wells
Version: Colls-437-05 03/09- ALPCO February 05, 2013
INTENDED USE

The ALPCO Type IV Collagen ELISA (Serum) provides a method for the quantitative determination of collagen IV in human serum. Please contact ALPCO for further information regarding the assay of collagen IV in other tissue fluids. The ALPCO Type IV Collagen ELISA is for research use only. Not for use in diagnostic procedures.

BACKGROUND

Chronic liver disease comprises a number of progressive disorders which culminate in liver cirrhosis and which are characterized by excessive deposition of collagen.

Although various types of collagen (type I, III, IV, V and VI) increase in the liver with the progression of fibrosis, type IV collagen, a constituent of the basement membrane, is particularly noteworthy for the following reasons: its serum level correlates with hepatic levels of collagen IV\(^1\), serum levels of collagen IV fall in response to effective therapy\(^1\) and it is the earliest type of collagen to be synthesized during experimental liver injury\(^2, 3\). Serum collagen IV levels are elevated in a variety of liver diseases\(^4-6\), in particular, serum collagen levels have been found to be predictive of therapy response in Hepatitis C infection\(^7\), and to be sensitive indicators of therapy response in abstaining alcoholics\(^1\).

ASSAY PRINCIPLE

The ALPCO Type IV Collagen ELISA is designed for the assay of serum collagen IV. It is a solid phase one-step sandwich ELISA. Collagen IV in the sample is bound simultaneously by a solid-phase monoclonal antibody and a monoclonal antibody-enzyme conjugate, each directed at different antigenic sites. This results in the collagen IV molecule being sandwiched between the solid phase and enzyme-labelled antibodies. After removing unbound enzyme-labelled antibody and sample, the plate is incubated with enzyme substrate. The resultant colour development is directly proportional to the amount of collagen IV in the sample.
COMPONENTS

1. Antibody coated Microassay plate: 12x8 well strips coated with IgG directed against human collagen IV. READY TO USE

2. Collagen IV Calibrator: Purified collagen IV in phosphate buffer (pH 7.0) with bovine serum albumin. 1000 μg/L Stock Solution (1mL). Contains 0.015% Geneticin as preservative. STOCK

3. Dilution Buffer: Phosphate buffer (pH 7.0) containing bovine serum albumin and horse serum (5mL). Contains 30 mg/L Proclin 300 preservative. READY TO USE

4. Conjugate: Anti-collagen IV mouse Fab’ conjugated to horseradish peroxidase (20 mL). Contains 30 mg/L Proclin 300 as preservative. READY TO USE

5. Wash Concentrate: 10x Conc. Phosphate buffer with Tween 20 (PBT), (2 bottles of 50 mL). Contains 30 mg/L Proclin 300 as preservative. CONCENTRATE

6. Substrate: Stabilized liquid TMB solution (15 mL). READY TO USE

7. Stop Solution: 1M Sulphuric Acid (15 mL) READY TO USE

8. Plate Seal: 1 sheet

9. Instructions for use

10. Uncoated microassay plate
PRECAUTIONS

SAFETY
- The ALPCO Serum Collagen IV ELISA kit is intended for use by qualified laboratory staff only.
- The kit contains material of human origin that has been tested and found to be negative for Hepatitis B surface antigen, Hepatitis C and HIV antibodies. However, since no test can provide complete assurance, treat all materials as potentially infectious.
- The Stop Solution contains sulphuric acid which is corrosive. Avoid contact with the skin and eyes. If contact occurs, rinse off immediately with water and seek medical advice.
- The Substrate contains TMB that may irritate the skin and mucous membranes. Any substrate which comes in contact with the skin should be rinsed off with water.
- Dispose of all clinical specimens, infected or potentially infected material in accordance with good laboratory practice. All such materials should be handled and disposed of as though potentially infectious.
- Residues of chemicals and kit components are generally considered as hazardous waste. All such materials should be disposed of in accordance with established safety procedures.
- Wear protective clothing, disposable latex gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
- Do not pipette materials by the mouth and never eat or drink at the laboratory workbench.

PROCEDURAL
- Do not use kit or individual reagents past their expiry date.
- Do not mix or substitute reagents from different kit lot numbers.
- Deviation from the protocol provided may cause erroneous results.
- Performing the assay outside the time and temperature ranges specified may produce invalid results. Assays not falling within the established time and temperature ranges must be repeated.
- Reagent delivery should be aimed at midpoint of the side of the wells, taking care not to scratch the side with the pipette tip.
- Do not allow the wells to dry at any stage during the assay procedure.
- Care must be taken not to contaminate components and always use fresh pipette tips for each sample and component.
- Do not use reagents that are cloudy or that have precipitated out of solution.
- Ensure Wash Concentrate is mixed thoroughly and no crystals remain before reconstitution.
- High quality distilled or deionised water is required for the Wash Solution. The use of poor quality or contaminated water may lead to background color in the assay.
- Allow all reagents to come to room temperature (20-27°C) and mix well prior to use.
- Avoid leaving reagents in direct sunlight and/or above 2-8°C for extended periods.
- Always use clean, preferably disposable, glassware for all reagent preparation.
- Ensure that the bottom surface of the plate is clean and dry before reading.
- Before commencing the assay, an identification and distribution plan should be established.
STABILITY AND STORAGE

1. All kit reagents should be stored at 2-8°C and are stable as supplied until the expiry date shown.
2. Prepared Wash Solution (PBT) is stable for up to one month at 2-8°C.
3. Prepared Calibrator solutions should not be stored.
4. Plate assay wells should be stored in sealed bags with desiccant at 2-8°C until required for use. Return unused wells to the storage bag together with desiccant.

ADDITIONAL MATERIALS REQUIRED

1. 20 μL and 150 μL micropipettes and a 100-150 μL multichannel pipette
2. Microassay strip washing system
3. ELISA plate reader capable of measuring at 450nm with reference at 630nm if available
4. 1 L beaker
5. Timer
6. Liquid trough
7. Deionised/Distilled water
8. Graduated cylinder (500 mL)

PREPARATION OF REAGENTS

WASH SOLUTION (PBT)
Perform a 1/10 dilution of Wash Concentrate adding, for example, 10 mL Wash Concentrate to 90 mL deionised water as required. Prepare only the volume of Wash Solution required for the assay. Each row of assay wells requires 10 mL of Wash Solution.

Ensure salt crystals are dissolved prior to dilution.
Gentle warming of Wash Concentrate at 37°C for 30 minutes will aid dissolution of salt crystals.

CALIBRATORS Using Labeled tubes prepare Calibrators as follows:

<table>
<thead>
<tr>
<th>Collagen IV Concentration (μg/L)</th>
<th>Calibrator Volume (μL)</th>
<th>Dilution Buffer (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 (A)</td>
<td>150 (A) (Stock Solution)</td>
<td>-</td>
</tr>
<tr>
<td>500 (B)</td>
<td>150 (A)</td>
<td>150</td>
</tr>
<tr>
<td>250 (C)</td>
<td>150 (B)</td>
<td>150</td>
</tr>
<tr>
<td>125 (D)</td>
<td>150 (C)</td>
<td>150</td>
</tr>
<tr>
<td>62.5 (E)</td>
<td>150 (D)</td>
<td>150</td>
</tr>
<tr>
<td>31.2 (F)</td>
<td>150 (E)</td>
<td>150</td>
</tr>
<tr>
<td>15.6 (G)</td>
<td>150 (F)</td>
<td>150</td>
</tr>
<tr>
<td>0 (H)</td>
<td>-</td>
<td>150</td>
</tr>
</tbody>
</table>

Calibrators should be prepared immediately before use. Do not store. The diluted calibrators are stable for at least 6 hours at 2-8°C.

SAMPLE HANDLING AND STORAGE

Samples can be stored at 2-8°C for one week. Samples may be stored at –20°C for 12 months. Repeated freeze-thawing of samples should be avoided.
ASSAY PROCEDURE

NOTE: All reagents should be allowed to reach room temperature prior to commencement of assay.

1. MIXING OF CALIBRATOR/SAMPLE
1.1 Prepare Wash Solution and Calibrators as described in "Preparation of Reagents".
1.2 Add Calibrators (H-A: 0 – 1000 μg/L) and samples (20 μL/well), in duplicate, to the uncoated Vinyl Microassay plate.
1.3 Add 150 μL Conjugate to each well.

2. IMMUNOREACTION
2.1 Place required number of anti-collagen IV coated Microassay wells in the assay plate (16 for the Calibrators plus two each for the samples).
2.2 Transfer 100μL of the mixtures from above into the equivalent wells in the anti- Collagen IV coated Microassay wells.
2.3 Cover the Microassay plate with the lid and incubate at room temperature (20- 27°C) for exactly 30 minutes.
2.4 Remove the plate lid and wash each strip three times (350 μL/well) with Wash Solution. When complete, firmly tap the plate against a paper towel to ensure complete removal of wash fluid from wells.

3. COLOR DEVELOPMENT
3.1 Add 100 μL Substrate/well using a multichannel pipette and incubate at room temperature (20-27°C) for exactly 30 minutes.

4. STOP
4.1 Add 100 μL Stop Solution/well using a multi channel pipette. Ensure complete mixing of Substrate and Stop Solution.
4.2 Read immediately at 450nm using 630nm as reference (if available).

CALCULATION OF RESULTS
1. Calculate the mean absorbance for each calibrator and sample.
2. Plot a Calibration curve of A450/630nm versus collagen IV (μg/L) on a log-log scale.
3. Read the collagen IV (μg/L) indicated by the mean absorbance’s of the samples from the calibration curve.
4. If the samples have been diluted, multiply the calculated [collagen IV] by the appropriate dilution factor in order to obtain the actual [collagen IV].
5. Concentrations of samples with readings outside the standard curve are invalid and must be repeated with a higher dilution factor. It is not acceptable to extrapolate data.
PERFORMANCE CHARACTERISTICS

EXPECTED RANGE
Based on healthy Japanese volunteers, the reference normal range for Collagen IV is: 99 + 23 µg/L. Mean + 1S.D. (N = 180).

LIMIT OF DETECTION
The detection limit of the Type IV Collagen ELISA is 15.6 µg/L.

MEASURING RANGE
The calibration curve range covers the range 15.6-1000 µg/L. This range may be extended by increasing sample dilution.

SPECIFICITY
The ALPCO Type IV Collagen ELISA is highly specific for the detection of collagen IV. Cross reactivity is less than 2% with Collagen II and less than 0.5% with other forms of collagen.

SENSITIVITY
When reading from the standard curve the $A_{450nm}$ value of the 1000 µg/L standard should be >0.6 OD.

INTERFERENCE
No significant interference has been observed in this assay with lipaemic, haemolytic or icteric samples.
- Lipaemia: Less than 10% interference up to 1200 Formazine turbidity units.
- Haemolysis: Less than 10% interference up to 3 g/L haemoglobin.
- Icteric: Less than 10% interference up to 0.2 g/L bilirubin.

DILUTION - RECOVERY
Dilution of samples containing high levels of collagen IV gave the following results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Expected µg/L</th>
<th>Obtained µg/L</th>
<th>Recovery %</th>
<th>Expected µg/L</th>
<th>Obtained µg/L</th>
<th>Recovery %</th>
<th>Expected µg/L</th>
<th>Obtained µg/L</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>57</td>
<td>61</td>
<td>107</td>
<td>28</td>
<td>32</td>
<td>114</td>
<td>14</td>
<td>16</td>
<td>114</td>
</tr>
<tr>
<td>B</td>
<td>107</td>
<td>110</td>
<td>103</td>
<td>53</td>
<td>58</td>
<td>109</td>
<td>27</td>
<td>28</td>
<td>104</td>
</tr>
<tr>
<td>C</td>
<td>259</td>
<td>270</td>
<td>104</td>
<td>130</td>
<td>139</td>
<td>107</td>
<td>65</td>
<td>67</td>
<td>103</td>
</tr>
</tbody>
</table>

REPRODUCIBILITY
Intra-assay variation of the Type IV Collagen ELISA

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\bar{x}$ [Collagen IV] µg/L</th>
<th>SD</th>
<th>%CV</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>119</td>
<td>7.4</td>
<td>6.2</td>
<td>8</td>
</tr>
<tr>
<td>Medium</td>
<td>218</td>
<td>7.8</td>
<td>3.6</td>
<td>8</td>
</tr>
<tr>
<td>High</td>
<td>520</td>
<td>12</td>
<td>2.3</td>
<td>8</td>
</tr>
</tbody>
</table>
Inter-assay variation of the Type IV Collagen ELISA

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\overline{x}$ [Collagen IV] µg/L</th>
<th>SD</th>
<th>%CV</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>115</td>
<td>11</td>
<td>9.6</td>
<td>6</td>
</tr>
<tr>
<td>Medium</td>
<td>291</td>
<td>13</td>
<td>4.5</td>
<td>6</td>
</tr>
<tr>
<td>High</td>
<td>370</td>
<td>31</td>
<td>8.2</td>
<td>6</td>
</tr>
</tbody>
</table>

Inter-batch Variation of the Type IV Collagen ELISA calculated for three batches of kits

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\overline{x}$ [Collagen IV] µg/L</th>
<th>SD</th>
<th>%CV</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>115</td>
<td>5</td>
<td>4.3</td>
<td>3</td>
</tr>
<tr>
<td>Medium</td>
<td>270</td>
<td>6</td>
<td>2.2</td>
<td>3</td>
</tr>
<tr>
<td>High</td>
<td>386</td>
<td>16</td>
<td>4.1</td>
<td>3</td>
</tr>
</tbody>
</table>

**EXAMPLE OF CALIBRATION CURVE**

![Typical Calibration curve](image)

Figure 1: Typical Calibration curve obtained using the Collagen IV ELISA. Plot of A450/630 nm versus [Collagen IV] µg/L. Assay range is 15.6 – 1000 µg/L.
REFERENCES

1) Tsutsumi, M. et al. (1996). Serum biomarkers for hepatic fibrosis in alcoholic liver disease: which is the better biomarker, type III procollagen, type IV collagen, laminin, tissue inhibitor of metalloprotease or prolyl hydroxylase? Alcoholism: Clinical and Experimental Research. 20(9), 1512-1517.


