Vitamin A & Vitamin E HPLC Assay

Catalog Number: VAE31-H100
100 Tests
For Research Use Only. Not for use in diagnostic procedures.

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1. Intended purpose

The Eagle Biosciences Vitamin A & Vitamin E HPLC Assay kit is intended for the quantitative determination of vitamin A and vitamin E in plasma and serum. The Vitamin A & Vitamin E HPLC Assay kit is for research use only and not to be used in diagnostic procedures.

2. Introduction

Vitamin A and E are fat soluble vitamins. Both are stored in the body, thus overdosing is possible. Vitamin A plays an important role in the visual process. It is mainly taken up as pre vitamin A by nutrition. In the liver it is processed to vitamin A. Retinal is involved in the visual process as prosthetic group of the rhodopsin molecule. Beside this, vitamin A (Retinol) is important for the growth of children and the growth of skin and mucous membranes. As an antioxidant, retinol protects the body against free radicals and reactive oxygen species. Overdosing leads to headache, vertigo, sickness and vomit. Disturbance of the nervous system, skin disease and loss of hair have also been reported.

Vitamin E protects the fatty acids against oxidation. It captures free radicals and reactive oxygen species. A lack of vitamin E is recognized in lipid metabolism disorders, liver disease and in early born children. It leads to muscle dystrophia, anemia and disturbances in the nervous system.

The Eagle Biosciences Vitamin A & Vitamin E HPLC Assay kit makes it possible to determine both vitamins in an easy, fast and precise method. The Eagle Biosciences Vitamin A & Vitamin E HPLC Assay kit includes all reagents ready to use for preparation and separation of the samples with exception of the columns (IC1600rp) and the controls (IC1600ko). Both can be supplied by Eagle Biosciences. Beside the complete test kits it is possible to order all components separately. Please request our single component price list.

3. Warnings and precautions

- All reagents of the Vitamin A & Vitamin E HPLC Assay kit are strictly intended for research use only and are not to be used for diagnostic procedures.
- Test kit and column are concerted. Using alternative columns might cause in insufficient separation, resulting in false high results. The given test characteristics might not be fulfilled.
- Do not interchange Vitamin A & Vitamin E HPLC Assay kit components from different lots.
- Calibrator and controls contain human blood. It was tested and found negative for
HBsAg, anti-HIV-1/2, and anti-HCV. No test can guarantee the absence of HBsAg or HIV, and so all human serum based reagents in this kit must be handled as though capable of transmitting infection.

- The internal standard, precipitating reagent and mobile phase contain organic solvents and have to be handled carefully. Organic solvents are highly flammable and toxic if inhaled or contact the skin. It should be handled with gloves, eye protection, and appropriate protective clothing in a hood. Any spill should be wiped out immediately with copious quantities of water. Do not breathe vapor and avoid inhalation. In case of an accident or indisposition contact immediately a physician.

- Wear disposable gloves while handling specimens or kit reagents and wash hands thoroughly afterwards.

- Do not pipette by mouth.

- Do not eat, drink, smoke or apply makeup in areas where specimens or kit reagents are handled.

- Reagents should not be used beyond the expiration date shown on kit label.

- Observe the guidelines for performing quality control in medical laboratories by assaying controls and/or pooled sera. During handling of all kit reagents, controls and serum samples observe the existing legal regulations.

### 4. Materials Provided

<table>
<thead>
<tr>
<th>Article no.</th>
<th>Component</th>
<th>Designation</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC1600lm</td>
<td>ELU</td>
<td>Mobile phase</td>
<td>1000 ml</td>
</tr>
<tr>
<td>IC1600st</td>
<td>STD</td>
<td>Standard, (concentration is given on the label)</td>
<td>10 ml</td>
</tr>
<tr>
<td>IC1600is</td>
<td>IS</td>
<td>Internal standard</td>
<td>5 ml</td>
</tr>
<tr>
<td>IC1600fr</td>
<td>PREC</td>
<td>Precipitation reagent</td>
<td>50 ml</td>
</tr>
<tr>
<td>IC1600vl</td>
<td>DIL</td>
<td>Dilution solution</td>
<td>10 ml</td>
</tr>
</tbody>
</table>
5. Additional special equipment

- Vortex mixer
- 1.5 ml reaction tubes (Eppendorf)
- Various pipettes
- HPLC with UV-detector
- HPLC column Vitamin AE (IC1600rp)
- Centrifuge

6. Reagent preparation

All test reagents are ready to use. The standard should be stored at -20 °C. All other test reagents should be stored at 2-8 °C up to the date of expiry stated on the label.

7. Specimen

- Plasma and serum could be used in this Vitamin A & Vitamin E HPLC Assay kit.
- Vitamin A and E are light- and temperature sensitive; therefore samples have to be protected from light, cooled and centrifuged immediately.
- The plasma and serum samples are stable in the dark at 2-8°C for minimum of 12 hours (vitamin A) and minimum of 3 days (vitamin E). At -20 °C vitamin A is stable for a minimum of 1 month and vitamin E for minimum of 3 month.

8. Procedure

Principle of the method

For the determination of vitamin A and E, an internal standard and the precipitation reagent are added. During the precipitation step, high molecular substances are removed. After centrifugation the supernatant is injected into the HPLC system. The isocratic separation via HPLC at 30°C uses a “reversed phase” column. One run lasts 15 minutes. The chromatograms are recorded by a UV-detector by two different wavelength (Vitamin A at 325 nm, Vitamin E 300 nm). The quantification is performed with the delivered plasma calibrator; the concentration is calculated by the “internal standard method” via integration of the peak heights resp. peak areas.
Sample and standard preparation

1. Pipette into 1.5 ml reaction tubes:

   - **Preparation of the standard:**
     - 250 µl STD
     - +
     - 50 µl IS
     - +
     - 250 µl DIL
     - +
     - 250 µl PREC

   - **Preparation of samples and controls:**
     - 250 µl sample or CTRL
     - +
     - 50 µl IS
     - +
     - 500 µl PREC

2. Vortex briefly. Leave the tubes for **30 minutes at 2-8°C** and centrifuge at 10,000g for 2 minutes.

3. Inject **100 µl** of the supernatant into the HPLC-system
Chromatographic conditions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column material:</td>
<td>Nucleosil® C18, 10 µm</td>
</tr>
<tr>
<td>Column dimension:</td>
<td>125 mm x 4 mm</td>
</tr>
<tr>
<td>Flow rate:</td>
<td>0.8 ml/min</td>
</tr>
<tr>
<td>UV-detection:</td>
<td>Vitamin A: 325 nm</td>
</tr>
<tr>
<td></td>
<td>Vitamin E: 300 nm</td>
</tr>
</tbody>
</table>

Switch the wavelength after 7 min.

**Injection volume:** 100 µl

**Running time:** 15 min

**Temperature:** 30 °C

To avoid contamination of the next run, use eluent (ELU) for autosampler needle washing.
We recommend using a guard-column to enlarge column lifetime.

**Treatment of the HPLC column**
Leave the column in eluent (ELU) after analysis. Before use, the system should be equilibrated with approx. 20 ml eluent (ELU).

**11. Calculation of analytical results**

**Calculation**

\[
\text{Conc. sample} = \frac{\text{peak area patient} \times \text{conc. calibrator}}{\text{peak area calibrator}} \times F
\]

\[
F = \frac{\text{Peak area IS of the calibrator}}{\text{Peak area analyte of the calibrator}}
\]
10. Internal quality control

Reference intervals

Vitamin A: 316 – 820 µg/l
Vitamin E: 6.6 – 14.3 mg/l

We recommend that each laboratory should develop their own normal range. The values mentioned above are only for orientation and can deviate from other published data.

11. Validation data

Precision and reproducibility

**Intra-Assay CV:**
- Vitamin A: 1.0 % (0.45 mg/l) \([n=6]\)
- Vitamin A: 0.9 % (0.97 mg/l) \([n=6]\)
- Vitamin E: 1.9 % (10.1 mg/l) \([n=6]\)
- Vitamin E: 1.1 % (20.1 mg/l) \([n=6]\)

**Inter-Assay CV:**
- Vitamin A: 4.4 % (0.42 mg/l) \([n=6]\)
- Vitamin A: 3.7 % (0.90 mg/l) \([n=6]\)
- Vitamin E: 5.1 % (9.2 mg/l) \([n=6]\)
- Vitamin E: 4.5 % (18.5 mg/l) \([n=6]\)

Linearity
- Vitamin A: up to 20 mg/l
- Vitamin E: up to 100 mg/l

Detection limit
- Vitamin A: 0.01 mg/l
- Vitamin E: 1.0 mg/l

Recovery
- Vitamin A: 98.9 %
- Vitamin E: 101 %

13. Disposal

The mobile phase (ELU), precipitation reagent (PREC), internal standard (IS) and standard (STD) must be disposed as non-halogenated solvent. Please refer to the appropriate national guidelines.

14. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible reason</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>No signal</td>
<td>No or defect connection to evaluation system</td>
<td>Check signal cord and connection</td>
</tr>
<tr>
<td></td>
<td>Detector lamp is altered</td>
<td>Change lamp</td>
</tr>
<tr>
<td>No peaks</td>
<td>Injector is congested</td>
<td>Check Injector</td>
</tr>
<tr>
<td>Double peaks</td>
<td>Dead volume in fittings and / or column</td>
<td>Renew fittings and / or column</td>
</tr>
<tr>
<td>Contaminating peaks</td>
<td>Injector dirty</td>
<td>Clean injector</td>
</tr>
<tr>
<td></td>
<td>Contamination at the head of</td>
<td>Change direction of the</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>--------------------------------------------</td>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>the column</td>
<td>column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase</td>
<td></td>
</tr>
<tr>
<td>Air in the system</td>
<td>Degas pump</td>
<td></td>
</tr>
<tr>
<td>Autosampler vials contaminated</td>
<td>Use new vials or clean them with methanol</td>
<td></td>
</tr>
<tr>
<td>Broad peaks, tailing</td>
<td>Precolumn / column exhausted</td>
<td>Use new precolumn / column</td>
</tr>
<tr>
<td>Variable retention times</td>
<td>Drift in temperature</td>
<td>Use a column oven</td>
</tr>
<tr>
<td></td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td></td>
<td>System is not in steady state yet</td>
<td>Rinse system mobile phase for 15 min</td>
</tr>
<tr>
<td>Baseline is drifting</td>
<td>Detector lamp did not reach working temperature yet</td>
<td>Wait</td>
</tr>
<tr>
<td></td>
<td>Detector lamp is too old</td>
<td>Renew lamp</td>
</tr>
<tr>
<td>Continue baseline is drifting</td>
<td>System is not in steady state yet</td>
<td>Rinse system mobile phase for 15 min</td>
</tr>
<tr>
<td></td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td>Baseline is not smooth</td>
<td>Pump delivers imprecise</td>
<td>Check pump, degas the system</td>
</tr>
<tr>
<td></td>
<td>Detector flowcell is dirty</td>
<td>Clean flow cell</td>
</tr>
</tbody>
</table>

15. Literature references


For further information about this kit, its application or the procedures in this kit insert, please contact the Technical Service Team at Eagle Biosciences at info@eaglebio.com or at 866-411-8023.