



EAGLE
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Vitamin A & Vitamin E HPLC Assay

Catalog Number: VAE31-H100

100 Tests

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0

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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

Article no.	Component	Designation	Amount
IC1600lm	ELU	Mobile phase	1000 ml
IC1600st	STD	Standard, (concentration is given on the label)	10 ml
IC1600is	IS	Internal standard	5 ml
IC1600fr	PREC	Precipitation reagent	50 ml
IC1600vl	DIL	Dilution solution	10 ml



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

Column material:	Nucleosil® C ₁₈ , 10 µm
Column dimension:	125 mm x 4 mm
Flow rate:	0.8 ml/min
UV-detection:	Vitamin A: 325 nm Vitamin E: 300 nm

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

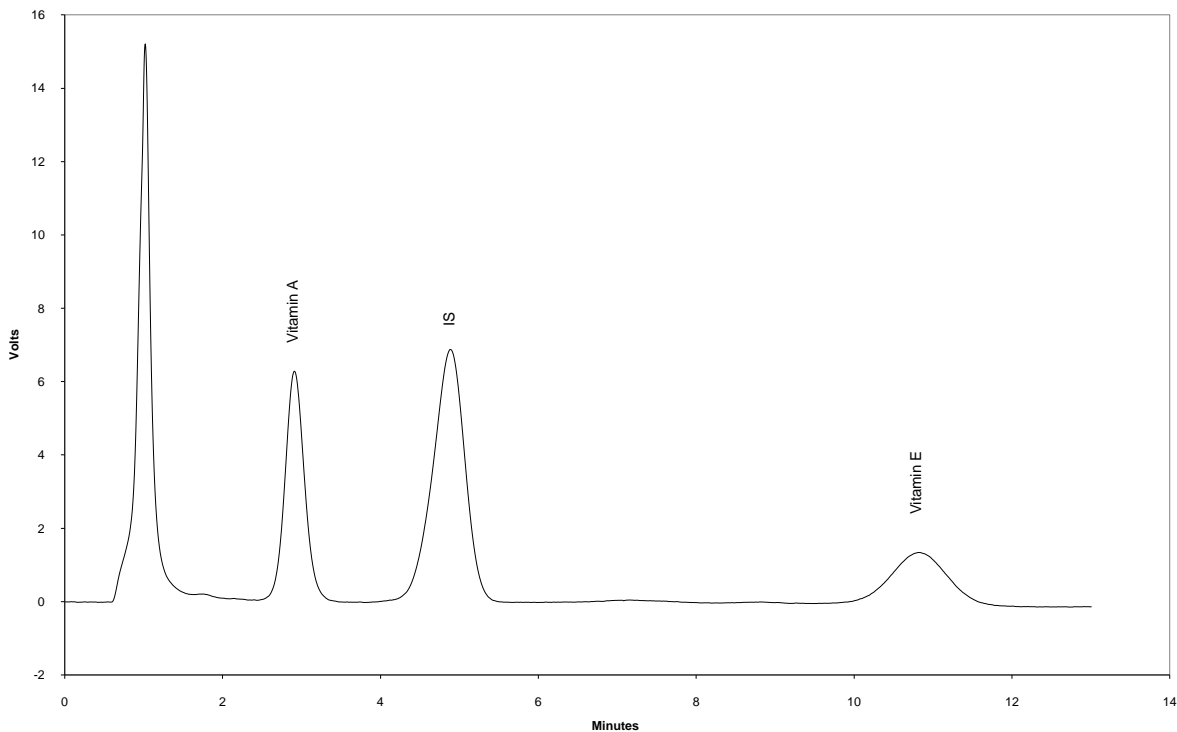
Calculation

$$\text{Conc. sample} = \frac{\text{peak area patient} * \text{conc. calibrator}}{\text{peak area calibrator}} * F$$

$$F = \frac{\text{Peak area IS of the calibrator}}{\text{Peak area analyte of the calibrator}}$$



Typical chromatogram



10. Internal quality control

Reference intervals

Vitamin A: 316 – 820 $\mu\text{g/l}$

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.

Source: B. Herbeth et al.; Clin Chem 32/9, 1756-1759 (1986)



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

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connection
	Detector lamp is altered	Change lamp
No peaks	Injector is congested	Check Injector
Double peaks	Dead volume in fittings and / or column	Renew fittings and / or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of	Change direction of the



	the column	column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas pump
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Use new precolumn / column
Variable retention times	Drift in temperature	Use a column oven
	Pump delivers imprecise	Check pump, degas the system
	System is not in steady state yet	Rinse system mobile phase for 15 min
Baseline is drifting	Detector lamp did not reach working temperature yet	Wait
	Detector lamp is too old	Renew lamp
Continue baseline is drifting	System is not in steady state yet	Rinse system mobile phase for 15 min
	Pump delivers imprecise	Check pump, degas the system
Baseline is not smooth	Pump delivers imprecise	Check pump, degas the system
	Detector flowcell is dirty	Clean flow cell

15. Literature references

- Sushil K.J., Mc Coy B., Wise R. (1994). Vitamin E and the hypercoagulability of neonatal blood. Clin Cim Acta 225; 97-103.
- Comstock G.W., Alberg A.J., Helzlsouer K.J. (1993). Reported effects of long-term freezer storage on concentrations of retinol, β -carotene, and α -tocopherol in serum or plasma summarized. Clin Chem 39/6; 1075-1078.

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.