Vitamin K1 HPLC Assay

Catalog Number: VK131-H100
100 Tests
For Research Use Only.
v. 2.0 (09.07.22)

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INTENDED PURPOSE

The Eagle Biosciences Vitamin K1 HPLC Assay is intended for the quantitative determination of vitamin K1 in plasma and serum. The Vitamin K1 HPLC Assay is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Vitamin K was first discovered by Dam in 1929. He fed chicken with ether extracted food and recognized deadly bleeding. Chicken supplemented with the ether extract normalized the symptoms. The hypothetic coagulation factor was called vitamin K. Vitamin K1 (phylloquinone) is synthesized by green plants and taken up by nutrition, whereas vitamin K2 (menaquinone) is produced by bacteria in the gut.

Vitamin K works as cofactor of an oxygen dependent carboxylase, which carboxylates specific glutamic acid residues in γ-position. Because of that modification, blood clotting factors can be activated by cleavage. In bone metabolism the γ-carboxylation enables osteocalcin to bind to hydroxyapatite of the bone matrix. Vitamin K can be stored only for a short time, whereby deficiency occur after a view days. A lack of vitamin K mainly manifests in clotting disorders. Beside this, disorders in bone metabolism caused by a deficient modification of osteocalcin have been reported.

The Eagle Biosciences Vitamin K1 HPLC Assay kit makes it possible to determine the vitamin in an easy, fast and precise method. The kit includes all reagents for the preparation and separation of the samples with exception of the columns (IC2400rp) and the controls (IC2400ko). Both can be supplied by Eagle Biosciences. Beside the complete test kit it is possible to order all components separately. Please request our single component price list.

WARNINGS AND PRECAUTIONS

- All reagents of the Vitamin K1 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 K1 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 mobile phase (ELU), internal standard (IS), isopropanolic vitamin K solution (ISOP), precipitation reagent (PREC) and extraction solution (EXTR) 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 Vitamin K1 HPLC Assay 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.

MATERIALS PROVIDED

Article no.	Component	Designation	Amount
IC2400lm	ELU	Mobile phase	3 x 1000 ml
IC2400ka	CAL	Calibrator, (lyoph. 2.2 ml)	8 vials
IC2400is	IS	Infernal standard	1 ml
IC2400il	ISOP	Isopropanolic Vitamin K1 solution	10 ml
IC2400fr	PREC	Precipitation reagent	200 ml
IC2400ex	EXTR	Extraction solution	400 ml
IC2400zi	ZINC	Zinc **(Important:** store under argon**)**	20 g
IC2400zn	Access	Accessories for post-column reduction reactor	5 pieces

The starter kit contains a post-column reduction reactor (empty).

MATERIALS NEEDED BUT NOT PROVIDED

- glass tubes for centrifugation, V-bottom (10 ml)
- SPE cartridges C18 from Waters WATO54945 (ordering no.: IC2400ck)
- Evaporator
- Centrifuge
- Various pipettes
- HPLC with Fluorescence-detector
- HPLC column Vitamin K1 (IC2400rp)
- Vortex mixer

REAGENT PREPARATION

Preparation of the Calibrator

- Reconstitute the **calibrator (CAL)** in **2.2 ml** deionized water. One vial is for **single use only**; discard the rest of the material. The concentration of vitamin K1 might have minor changes from lot to lot.
- All other test reagents are ready to use and stable at 2-8 °C, up to the date of expiry stated on the label.

Preparation of the Post-Column Reduction Reactor

- Vitamin K is reduced during the passage through the post column reactor. The reactor has to be filled new before each batch of samples, because the surface of the zinc particles is oxidized after 12 hours running time. Filling the column should take 10 min.
- The reactor is assembled in the following order
    1. Cap nut
    2. Stainless steel inlet
    3. PTFE seal
    4. Stainless steel sieve (grey)
    5. Glass fiber sieve (3 pieces, white)
    6. Stainless steel sieve (grey)
    7. Column tube

- First one side of the reactor is closed.
- Fill in the zinc-particles with a funnel while knocking the column slightly on the table, so that the packing will be homogenous.
- Close the other side of the column.
- The post-column reduction reactor should be mounted in the HPLC-system between the HPLC column and the detector inlet.

SPECIMEN

- EDTA-plasma and serum can be used.
- Vitamin K1 is temperature sensitive; therefore samples have to be cooled immediately.
- The samples are stable in the dark at 2-8°C for 1 week. For longer storage samples should be frozen at -20 °C.

PROCEDURE

PRINCIPLE OF THE METHOD

For the determination of vitamin K1 an internal standard is added first. After a solid phase extraction on SPE-cartridges, the samples are precipitated. The supernatant is then extracted with an organic solvent and evaporated. After that the samples are re-suspended and injected into the HPLC system. A post-column reduction reactor reduces vitamin K and enables the measurement of vitamin K with a fluorescence detector. The isocratic separation at 30°C using a "reverse phase" column lasts 20 minutes. The chromatograms are recorded by a fluorescence

detector. The quantification is performed by the delivered plasma calibrator; the concentration is calculated by the internal standard method via integration of the peak areas resp. heights.

SAMPLE PREPARATION

1. The cartridge is rinsed with 3 ml methanol and 3 ml deionized water.

   1 ml sample, CAL or CTRL

   +

   10 µl IS

2. Pipette the mixture on the SPE cartridge. Let it soak through by vacuum and collect the break-through in a glass vial.

3. Add **2 ml PREC** , vortex for 1 min. and centrifuge for 5 min. at 10.000 x g.

4. Pipette the supernatant in a fresh glass vial and add **4 ml** of **EXTR**.

5. Vortex for 2 min and centrifuge for 5 min at 3.500 x g.

6. Transfer the upper layer in a new glass vial and evaporate to dryness. **The dried sample is stable for 8 days at 4-8°C**

7. Connect the post-column reduction reactor in the HPLC-system, as described above and wait for equilibration (30-45 min).

8. Check the performance of the reactor by the injection of **100 µl** of **ISOP** and determine the signal to noise ratio, which should be greater than 25.

9. Re-suspend the dried sample in **150 µl ELU** and inject **100 µl** in the HPLC-system.

Chromatographic Conditions

Column Material:	Superspher® 100 RP 18, 4 µm	
Column Dimension:	125 mm x 4 mm	
Flow Rate:	1-1.2 ml/min	
Fluorescence Detection:	Excitation	248 nm
	Emission	418 nm
Injection Volume:	100 µl	
Running time:	20 min	
Temperature:	30 °C	

Important: The mobile phase (ELU) must not be re-circulated

Treatment of the HPLC Column

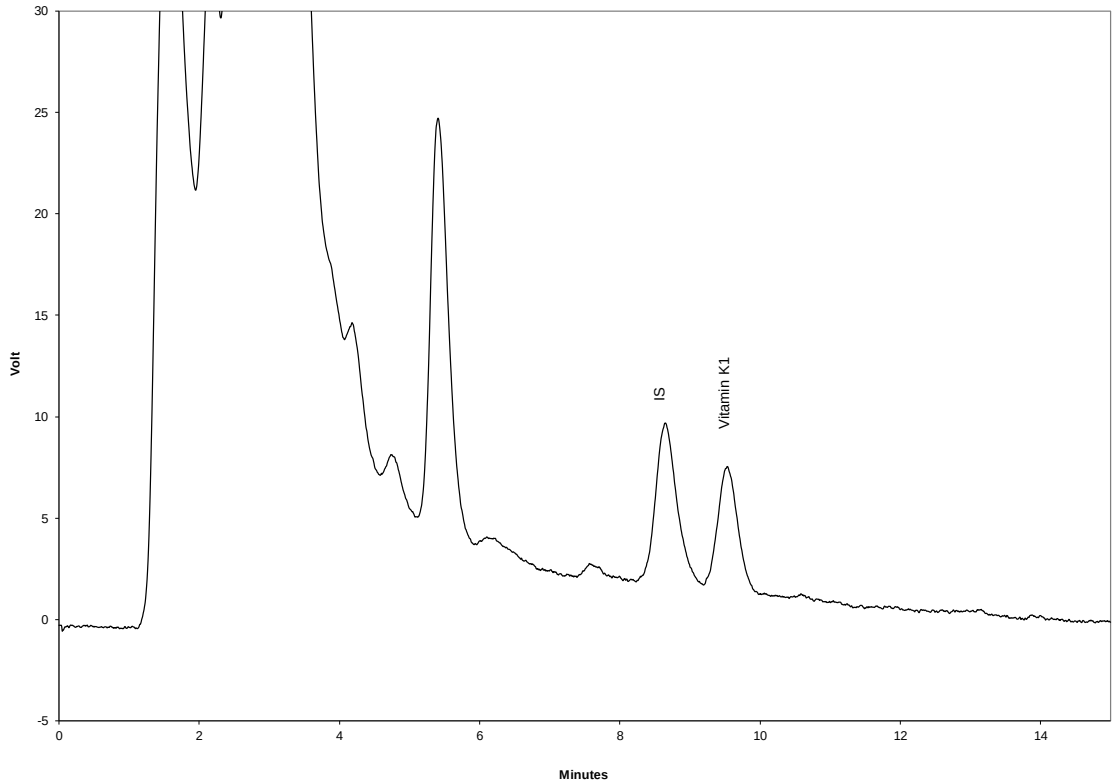
After the analysis the column should be flushed with 15 ml deionized water (1 ml/min) and stored in 50% methanol/ deionized water (approx. 15 ml, flow 0.7 ml/min). Before use, the system should be equilibrated with approx. 30 ml mobile phase (ELU).

CALCULATIONS

$$\frac{\text{peak area patient} \cdot \text{concentration of the standard}}{\text{peak area IS patient}} * F = \text{concentration patient sample}$$

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

Typical Chromatogram



INTERNAL QUALITY CONTROL

Reference Intervals

0.5 – 5 ng/ml

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.
(W. Friedrich Vitamins, de Gruyter 1988, ISBN 0-89925-273-7

VALIDATION DATA

Precision and Reproducibility

Intra-Assay CV:	2.5 % (0.77 ng/ml)	[n = 6]
	0.9 % (2.69 ng/ml)	[n = 6]
Inter-Assay CV:	6.9 % (0.85 ng/ml)	[n = 6]
	3.8 % (2.89 ng/ml)	[n = 6]

Linearity:

up to 25 ng/ml

Detection limit:

0.07 ng/ml

Recovery:

92.5 %

LIMITATIONS

Strong hemolytic and lipemic samples should not be measured.

DISPOSAL

The mobile phase (ELU), isopropanolic standard (ISOP), internal standard (IS), extraction solution (EXTR) and precipitating reagent (PREC) must be disposed as non-halogenated solvent.
Please refer to the appropriate national guidelines.

TROUBLESHOOTING

Problem	Possible reason	Solution
No signal	No or defect connection to evaluation system	Check signal cord and connections
	Lamp of detector is altered	Renew lamp
No peaks	Injector is congested	Check injector
Double peaks	Dead volume in fittings and/or at the head of column	Renew fittings and/or column
Contaminating peaks	Injector dirty	Clean injector
	Contamination at the head of the column	Change direction of the column and rinse for 30 min at low flow rate (0.2 ml/min) with mobile phase
	Air in the system	Degas the mobile phase and pump head
	Autosampler vials contaminated	Use new vials or clean them with methanol
Broad peaks, tailing	Precolumn / column exhausted	Renew 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
	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 flow cell is dirty	Clean flow cell
	Detector lamp is too old	Renew lamp

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