Product No: JSM-CL-2700

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Vitamin K in Serum/Plasma LC-MS/MS Analysis Kit

Vitamin K is a cofactor for enzymatic modification of glutamic acid residues (Glu) to gamma-carboxyglutamic acid residues (Gla) in vitamin K-dependent Gla proteins. These Gla proteins are necessary for hemostasis, bone metabolism, vascular calcification and cell proliferation. Vitamin K is found in nature as phylloquinone (vitamin K1) in green leafy vegetables, algae and some plant oils, and menaquinones (vitamin K2 also termed MK-n, where n is the number of isoprenoid units) can be found in meat, eggs and fermented food. A synthetic form of vitamin K menadione (vitamin K3) can be found as an additive in some animal feeds.

Highlights of the Analysis Kit

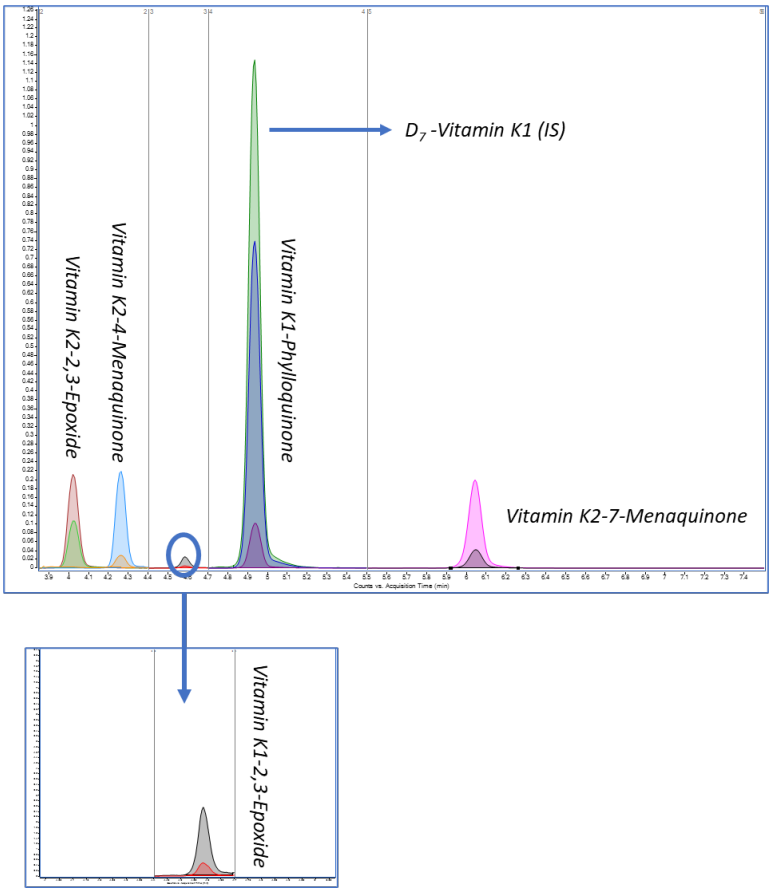
- Simultaneous measurement of five forms of vitamin K
- Total run time is 12 min.
- Liquid–liquid extraction with protein precipitation without SPE
- Long life span of HPLC column

Parameters		
Vitamin K1-Phylloquinone Vitamin K1-2,3-Epoxide	Vitamin K2-7-Menaquinone Vitamin K2-2,3-Epoxide	Vitamin K2-4-Menaquinone
Sample Type		
Serum/Plasma		

Sample Preparation

1	Pipette 400 µL of serum sample into a glass centrifuge tube
2	Add 800 µL of Reagent 1, additionally vortex for 5 sec.
3	Add 25 µL of internal standard, then 2 ml Reagent-2 and vortex for 3 sec.
4	Centrifuge at 4000 rpm for 5 min.
5	Transfer 1.5 ml of the upper phase to a new glass tube (tube-2) and evaporate under nitrogen stream
6	Reconstitute tube-2 with 100 µL of Reagent-3 and transfer to an amber HPLC vial prior to LC-MS/MS system

Example Chromatogram



Total ion chromatogram of Vitamin K forms

Method Performance

All validation results were obtained using Agilent 6470 systems

Analytes	LOQ (µg/L)	Linearity (µg/L)	Recovery (%)		Repeatability (%CV)	
			LLQC*	HLQC**	LLQC	HLQC
Vitamin K1-Phylloquinone	0.07	0.4 – 50	103	102	5.0	3.8
Vitamin K1-2,3-Epoxide	1.8	2 – 250	110	108	9.1	7.8
Vitamin K2-7-Menaquinone	0.37	0.4 – 50	99	102	6.2	4.5
Vitamin K2-2,3-Epoxide	1.5	2 – 250	105	102	7.1	5.3
Vitamin K2-4-Menaquinone	0.04	0.4 – 50	98	99	5.1	3.6

LLQC: Low-level quality control
** HLQC: High-level quality control

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