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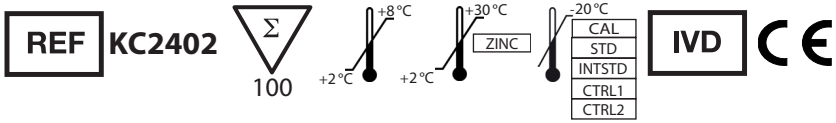
Short manual

For professional use only

IDK® Vitamin K_1/K_2 HPLC Kit

For the in vitro determination of vitamin K_1 and K_2 in plasma and serum

Valid from 2026-04-14



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Important note

The following instructions serve as a short manual for the preparation and execution of the test. **It is important to note that this document does not replace the full instructions for use.**

Table of Contents

1. INTENDED PURPOSE

The Immundiagnostik AG assay is intended for the quantitative determination of vitamin K_1 and K_2 in plasma and serum. For professional *in vitro* diagnostic use only.

2. INTRODUCTION

Vitamin K is rapidly metabolized in the body and stored only in small amounts, which is why a deficiency can develop even after a short period of time. Clinically, vitamin K deficiency manifests primarily as bleeding in the skin, mucous membranes, muscle tissue, and internal organs caused by impaired blood clotting, or as disturbances in bone metabolism resulting from insufficient carboxylation and activation of osteocalcin. Deficiency symptoms result from a reduced effect of vitamin K as a cofactor of a microsomal, O_2-dependent carboxylase, whose enzymatic function is crucial for the carboxylation of certain glutamic acid residues of coagulation factors, as well as of osteocalcin. Due to reduced formation of γ-carboxyglutamic acid residues, the coagulation factors lose their ability to form complexes with calcium ions (Ca^{2+}) and membrane phospholipids. These complexes are necessary for the release of active coagulation factors following proteolytic cleavage. Furthermore, due to insufficient γ-carboxylation, osteocalcin loses its ability to bind to calcium hydroxyapatite (CaHA) in the bone matrix, thereby impairing bone health. This ultimately leads to the typical symptoms of vitamin K deficiency. In severe cases, severe anemia may also occur due to hypoplastic changes in the bone marrow.

Indications

- Blood clotting disorders
- Bone metabolism disorders
- Vitamin K deficiency induced by:
  - obstructive liver disease
  - obstructive icterus
  - malabsorption due to celiac disease, pancreatitis, diarrhea
  - long-term antiobiotic therapy: Destruction of the intestinal flora
- Haemorrhagic disorders of newborns

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KC2402	CAL	Calibrator, lyophilised	4 x
	CTRL1	Control 1, lyophilised	4 x
	CTRL2	Control 2, lyophilised	4 x
	PREC	Precipitating reagent, ready-to-use	220 ml
	ELUSOL	Elution solution, ready-to-use	420 ml
KC2400	MOPHA	Mobile phase, ready-to-use (important: do not recirculate)	3 x 1 000 ml
	STD	Isopropanolic standard, ready-to-use	2 ml
	INTSTD	Internal Standard, ready-to-use	1 ml
	ZINC	Zinc	12 g
	ZUB	Accessories for post-column reduction reactor	1 x
K 0005.15	RECSOL	Reconstitution solution, ready-to-use	1 x 15 ml

The HPLC column (KC2400RP) and the post-column reduction reactor (KC2400NR) can be ordered separately from Immundiagnostik AG. To extend the lifetime of your HPLC column, pre-columns (KC2400VS) should ideally be used. These and also the corresponding pre-column holders (KC2400VH) can also be ordered from Immundiagnostik AG. In addition to the complete kit, all components as well as required materials (e.g. borosilicate glass tubes (KC0001V), evaporator (KC0001SV)) can be ordered separately. Please ask for our single component price list.

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Centrifuge
- Vortex mixer with no specific requirements
- 10 ml test tubes, e.g. round-bottom tubes (catalog number KC2402V)
- HPLC system with a fluorescence detector
- Post-column reduction reactor (catalog number KC2400NR)
- Reversed-phase C18-column (catalog number KC2400RP)
- Calibrated precision pipettes and disposable pipette tips with variable volumes of 100–1 000 µl

- Device for concentrating the sample (e.g. vacuum centrifuge or evaporator, catalog number KC0001SV)
- PEEK or stainless steel "union" and column connection capillary

5. STORAGE AND PREPARATION OF REAGENTS

Preparation of the calibrator and controls

- The calibrator **(CAL)**, the internal standard **(INTSTD)**, the controls **(CTRL1** and **CTRL2)**, and the isopropanol standard **(STD)** can be used until the expiration date (see label) when stored at **-20 °C**. The other components, with the exception of zinc and the filters/seals for the reactor, are stored at **2–8 °C**.
- The calibrator **(CAL)** and controls **(CTRL1** and **CTRL2)** are resuspended with **x µl** of reconstitution solution **(RECSOL)** (**x** = see product specification). The content of vitamins K_1 and K_2 varies slightly from batch to batch; the exact content is provided in the accompanying product specification.
- The calibrator **(CAL)** and controls **(CTRL)** are stable for at least one working day **(up to 12 hours)** at temperatures up to **28 °C** after reconstitution. Do not refreeze reconstituted, unused residues, as this leads to partial destruction of the calibrator.
- Before first use of the assay, transfer the entire contents of the internal standard **(INTSTD)** into the precipitation reagent **(PREC)** and mix thoroughly. The resulting mixture should be stored **protected from light** at **-20 °C to 8 °C** and can be used until the kit's specified expiration date. Mix thoroughly before each use.

Preparation of the mobile phase

If the HPLC system used does not have an in-line degasser, the mobile phase should be degassed in an ultrasonic bath for 10–15 minutes. Since the mobile phase is an organic solvent, it is important to seal the autosampler vials with a septum prior to injection into the HPLC system to prevent evaporation and ensure consistent measurement quality. Only suitable alternatives (e.g., PEEK frits) should be used to avoid material incompatibilities and interference with the analytical process.

System preparation

The method used here is known as "non-aqueous reverse-phase HPLC". Compared to a conventional RP-HPLC method, the following additional steps must be considered:

Removal of residues from aqueous methods:

- Replace the column with a Union
- Flush the entire system for at least 20–30 minutes with pure methanol at a flowrate of 1–2 ml/min.

Autosampler rinsing:

- Replace AS1 with pure methanol and rinse as previously described.
- Alternatively, rinses can be deactivated.

Column and post-column reduction reactor:

- After flushing the system and autosampler, connect the column as shown in the figure.
- The column is then flushed with mobile phase to remove the storage solution.
- Once the baseline stabilizes, replace the union with the post-column reduction reactor and flush for at least 15 minutes.

Preparation of the post-column reduction reactor

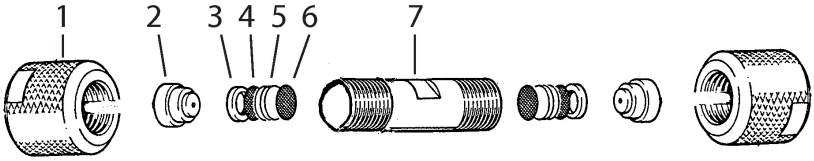
In the post-column reduction reactor, vitamins K_1 and K_2 are reduced, thereby making them detectable by the fluorescence detector. The loading process is straightforward and takes about 5 minutes.

Alternative procedure: The reactor can be removed together with the column and replaced with a union for rinsing. This allows the column and reactor to be reused (store at **2–8 °C**).

Important: Check the reactor's performance before each use. For details, see chapter 7 "Assay procedure - *Test procedure",* in the complete instructions for use.

For assembling the column see the following figure.

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



1. Close one side of the column according to the figure above.
2. Fill in the zinc powder with a funnel or spatula while knocking the column slightly on the table, so that the packing will not show any cavities.
3. Close the upper side of the column.

The post-column reduction reactor should be mounted in the HPLC-system as described by the following picture:

6. STORAGE AND PREPARATION OF SAMPLES

Plasma and serum are suitable as samples. The samples are stable for 7 days at room temperature (15–30 °C) when protected from light and can also be centrifuged 72 hours later at room temperature when protected from light. For longer storage, they should be frozen at -20 °C.

7. LIMITATIONS

We recommend not to measure hemolytic and lipaemic patient samples.

8. DISPOSAL

Mobile phase (**MOPHA**), isopropanolic standard (**STD**), internal standard (**INTSTD**), elution solution (**ELUSOL**) and precipitating reagent (**PREC**) must be disposed of as non-halogenated solvent. Please refer to the appropriate national guidelines.

9. PRECAUTIONS

- Human materials used in kit components were tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- The supplied reagents contain organic solvents. Although diluted, they still should be handled with care. It can cause burns and should be handled with gloves, eye protection and appropriate protective clothing. Do not breathe vapour and avoid inhalation.
- As a precaution, it is recommended that the human material used is always considered potentially infectious.

10. GENERAL NOTES ON THE TEST

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- Do not use reagents beyond the expiration date stated on the kit label.
- Do not mix different lot numbers of any kit component within the same assay.
- Do not mix plugs and caps from different reagents.
- Follow the guidelines for medical laboratories.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which has not been consulted with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from incorrect use.

- Warranty claims and complaints regarding deficiencies must be made within 14 days after receiving of the product. The product should be sent to Immundiagnostik AG along with a written complaint.
- Analyse controls with each run.
- Always perform the assay according to the enclosed manual.
- Serious incidents are to be reported to Immundiagnostik AG and the national regulatory authorities.
- ***IDK***® is a trademark of Immundiagnostik AG.

11. REFERENCES

1. Saupe J. (1992). Vitamin K und Indikationen zur Bestimmung im Plasma. Klin. Lab. 38: 51-54
2. Szulc P., Arlot M., Chapuy M.C., Duboef F., Meunier P.J., Delmas P.D. (1994). Serum undercarboxylated osteocalcin correlates with hip bone mineral density in elderly women. J Bone Miner Res 9:1591-95
3. Hodges S.J., Akesson K., Vergnaud P., Obrant K., Delmas P.D. (1993). Circulating levels of vitamin K1 and K2 decreased in elderly women with hip fracture. J Bone Miner Res 8:1241-45
4. Haroon Y, Bacon DS, Sadowski JA. (1986) Liquid-chromatographic determination of vitamin K1 in plasma, with fluorometric detection. Clin Chem. Oct;32(10):1925-9

12. SYMBOL EXPLANATION


Temperature limitation

Catalogue number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Content sufficient for <n> tests

Lot number


Use by


Contains plasma derivatives or human blood


Consult instructions for use

Consult specification data sheet


Do not re-use

Unique Device Identification

Contains material of animal origin


Medicinal substance

Contains material of human origin