



Li StarFish S.r.l.
Via Cavour, 35 - 20063 Cernusco S/N (MI), Italy
Tel. +39-02-92150794 - Fax. +39-02-92157285
info@listarfish.it - www.listarfish.it

**Manual
Preliminary**

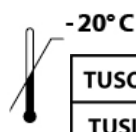
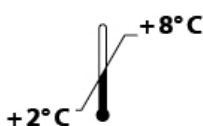
1,25-(OH)₂ Vitamin D₃/D₂ LC-MS/MS Tuning Kit

For determination of the optimal LC-MS/MS settings

Valid from 21.09.2011

REF

IMM-KM 1001



TUSOL
TUSIS

IVD



1. INTENDED USE

In preparation for the Immundiagnostik 1,25-(OH)₂-Vitamin D₃/D₂ LC-MS/MS application an instrument tuning is necessary. The tuning kit is intended for determination of the optimal LC-MS/MS settings as well as for testing the sensitivity of the instrument.

The MS/MS parameters recommended by Immundiagnostik AG are only for orientation and must be optimized for each instrument.

2. MATERIAL SUPPLIED

Cat. No	Label	Kit Components	Quantity
KM1000LA	MOPHA A	Mobile Phase A	1000 ml
KM1000LB	MOPHA B	Mobile Phase A	1000 ml
KM1000AC	ACTSOL	Activating reagent	2,5 ml
KM1001TU	TUSOL	Tuning solution 1,25-(OH) ₂ -Vitamin D ₃ /D ₂	5 ml
KM1002TU	TUSIS	Tuning solution Internal Standard	2 ml
KM1000SO	SOL A	Solution A	25 ml

The UPLC column with Inline Filter (KM1000RP) can be ordered separately from Immundiagnostik.

3. MATERIAL REQUIRED BUT NOT SUPPLIED

- Vortex mixer
- 2 ml glass vials, suitable for LC-MS/MS
- 500 ml Measuring cylinder, suitable for LC-MS/MS
- Different pipettors
- LC-MS/MS Instrument
- RP-C₁₈ column, e. g. Acquity BEH C18, 1,7 µm (2,1 x 50 mm)

4. PREPARATION AND STORAGE OF REAGENTS

Mobile phases:

Add 0.1% activation reagent (ACTSOL) to the mobile phases (MOPHA A, MOPHA B) and solution A (Sol A) before use:

e. g. 500 ml MOPHA (MOPHA A or MOPHA B) + 500 µl ACTSOL

The obtained solutions are stable for 2 weeks.

Warning: The activation reagent (ACTSOL) must be added under the fume hood. All vials to be used must be absolutely clean and detergent-free as well as preferentially made of glass suitable for LC-MS/MS analysis.

Tuning solutions (TUSOL, TUSIS) must be stored at -20°C and used until the expiration date given on the label. All other reagents are stable at 2-8 °C up to the date of expiry stated on the label.

5. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The mobile phases (MOPHA A and MOPHA B) as well as tuning solutions (TUSOL, TUSIS) and solution (SOL A) contain organic solvents and must be handled with gloves, eye protection, and appropriate protective clothing in a hood.
- The ACTSOL (activation reagent) consists of a strong acid and must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on kit label.

6. ASSAY PROCEDURE

Procedural notes

- The assay should always be performed according the enclosed manual.

6. 1.TUNING

We recommend tuning with a T-fitting (UPLC/HPLC flow rate and a syringe pump). The tuning solutions (TUSOL, TUSIS) consist of highly pure 1,25-(OH)₂-Vitamin D₃/D₂ or isotopically labeled 1,25-(OH)₂-Vitamin D₃-d₆, each with a concentration of 1 µg/ml.

HPLC/UPLC flow rate of activated MOPHA A: 0.2 ml/min

Flow rate of the syringe pump with a tuning solution: 10 -20 µl/min

MS/MS method (e. g. for a Waters Quattro Premier XE Tandem mass spectrometer):

Mode:	MRM
Polarity:	ESI+
Capillary (kV):	3
Cone (V):	var.
Extractor (V):	4
RF Lens (V):	0
Source Temperature (°C):	130
Desolvation Temperature (°C):	450
Cone Gas Flow (L/Hr):	50
Desolvation Gas Flow (L/Hr):	950
Collision Gas Flow (mL/Min):	0.15

MRM transitions (m/z):

1,25-(OH)₂-Vitamin D₃

399.11 > 134.58	Cone Voltage: 30	Collisions Energy: 22
399.11 > 150.64	Cone Voltage: 30	Collisions Energy: 22

1,25-(OH)₂-Vitamin D₂

411 > 134.8	Cone Voltage: 20	Collisions Energy: 23
411 > 150.7	Cone Voltage: 30	Collisions Energy: 26

Internal Standard / Isotopically labeled 1,25-(OH)₂-Vitamin D₃-d₆

405.21 > 134.68	Cone Voltage: 35	Collisions Energy: 20
405.21 > 150.62	Cone Voltage: 35	Collisions Energy: 23

1,25-(OH)₂-Vitamin D₃ has a molecular mass of 416.64 Da, 1,25-(OH)₂-Vitamin D₂ – 428.65 Da and isotopically labeled 1,25-(OH)₂-Vitamin D₃-d₆ – 422.65. The masses of 399.11 Da, 411 Da and 405.21 Da correspond to a loss of a molecule of water, respectively.

6.2. SENSITIVITY TEST

After obtaining a stable precursor ion and the corresponding fragment ions, a dilution series of the tuning solution are prepared to test the linearity and sensitivity of the LC-MS/MS system.

Preparation of the dilution series:

The tuning solution (TUSOL) is diluted with activated solution A (SOL A) as follows:

1.) Preparation of the highest concentration:

TUSOL 1:1000 in SOL A

e.g. 10 µl TUSOL in 9990 µl SOL A (1000 pg/ml)

2.) Preparation of the dilution series:

			Concentration [pg/ml]
1000 µl	1000 pg/ml		1000
500 µl	1000 pg/ml +	500 µl SOL A	500
500 µl	500 pg/ml +	500 µl SOL A	250
500 µl	250 pg/ml +	500 µl SOL A	125
200 µl	125 pg/ml +	800 µl SOL A	25
		1000 µl SOL A	Blank

50 µl of the samples can be injected directly in the LC-MS/MS system. A duplicate injection of the samples is recommended. For determination of the retention time, solely the highest concentration of 1000 pg/ml can be injected in the first step.

Chromatographic conditions:

Column material:	z. B. Acquity BEH C18; 1.7 µm		
Column dimension:	2.1 x 50 mm		
Flow rate:	0.3 ml/min		
Column temperature:	45°C		
Injection volume:	50 µl		
Run time:	3.5 min		
Gradient:	0 min	100% A	0% B
	2 min	0% A	100% B
	2.1 min	0% A	100% B
	2.2 min	100% A	0% B
	3.5 min	100% A	0% B

It is recommended that a guard column or inline-filter is used to extend column life.

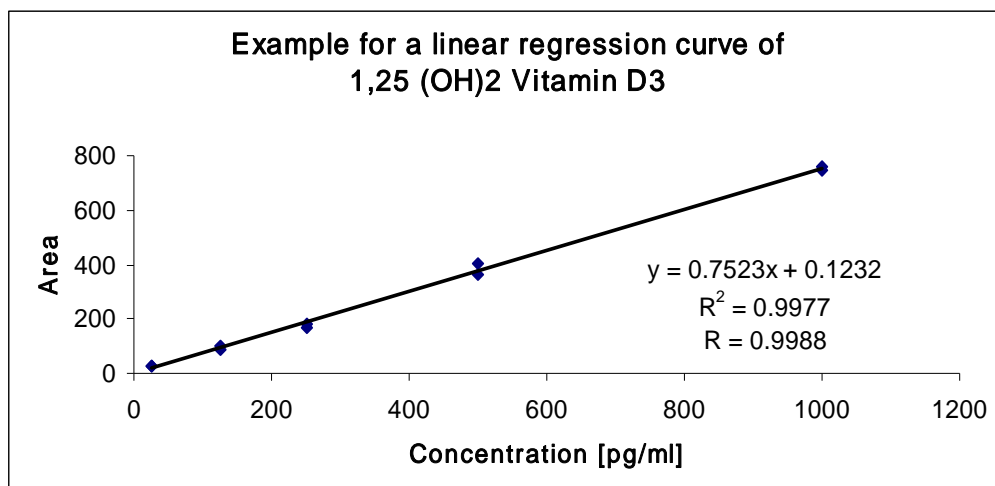
7. TREATMENT OF THE COLUMN

After the analysis, the column should be run with ca. 20 ml of 50% methanol stored in it.

8. RESULTS

Die obtained areas are plotted against the concentrations and a linear regression curve is generated. The lowest concentration of 25 pg/ml should be found to obtain the necessary sensitivity.

When the obtained results are satisfying, the 1,25-(OH)₂-Vitamin D₃/D₂-Immuno Tube® LC-MS/MS Kit of Immundiagnostik AG can be used for measurement of 1,25-(OH)₂-Vitamin D₃/D₂ in plasma and serum.



9. DISPOSAL

The mobile phases (MOPHA A, MOPHA B), activating reagent (ACTSOL) and Solution A (SOL A) must be disposed as non-halogenated solvents.

Please refer to the appropriate national guidelines.

10. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and distributed according to the IVD guidelines of 98/79/EC.
- The test components contain organic solvents. Contact with skin or mucous membranes must be avoided.
- All reagents in the test package are for *in vitro* diagnostic use only.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Do not interchange different lot numbers of any kit component within the same assay.

Used symbols:



Temperature limitation



Catalogue Number



In Vitro Diagnostic Medical Device



Contains sufficient for <n> tests



Manufacturer



Use by



Lot number

Li StarFish distribuisce: