

Monoclonal Antibodies to 25OH Vitamin D

Author:

Nicolas Heureux, PhD

Principal Scientist – Vitamin D, DIAsource Immunoassays

DIAsource ImmunoAssays S.A.

Rue du Bosquet, 2 | B-1348 Louvain-La-Neuve | Tel : +32.10.84.99.11 | Fax : +32.10.84.99.90
www.diasource.be

北京来福赛思科技有限公司 电话: 010 5701 7625 / 5701 8690 / 5701 8691 传真: 010 8174 3370

微电: 136 4103 4192 / 135 2194 3723 网站: www.bj-life-science.com 邮箱: info@bj-life-science.com



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1. INTRODUCTION

VITAMIN D

Since many years the role of vitamin D in bone and mineral metabolism was recognized in bone-related diseases. Clinical applications of 25OH Vitamin D measurements were merely related to the diagnosis and monitoring of therapy for rickets (children), osteomalacia, postmenopausal osteoporosis, and renal osteodystrophy. As a results of more recent studies a link between Vitamin D deficiency and many other diseases is suggested. These include cancer, cardiovascular disease, autoimmune diseases, diabetes, depression and many others.

There are two forms of Vitamin D in the human body namely Vitamin D3 (cholecalciferol) and Vitamin D2 (ergocalciferol), which are structurally very similar. Vitamin D3, the main form in humans, is produced in the skin from 7-dehydrocholesterol in response to direct sunlight and can also be obtained in small amounts from animal based foods (oily fish, primarily salmon and mackerel). Vitamin D2 can be obtained in small amounts from plant-based foods (some vegetables, yeast and fungi). Vitamin D3 and D2 are metabolized in the liver to their respective 25OH Vitamin D3 and D2 forms which are converted in the kidneys and in many tissues to the active forms (1,25(OH)₂ Vitamin D3 and D2).

DETERMINING VITAMIN D STATUS

The measurement of 25OH Vitamin D concentration in the serum or plasma is so far the best indicator of Vitamin D nutritional status. It is generally accepted that serum 25OH Vitamin D levels reflect the body's storage levels of Vitamin D and correlate with the clinical symptoms of Vitamin D deficiency. There is no consensus about the optimal 25OH Vitamin D level, but many publications suggest a range ≥ 30 ng/mL (>80 nmol/L) as optimal. The most widely used intervals are indicated in table 1.

Table 1 : Suggested Reference Values for Adults	
Vitamin D Status	25-Hydroxyvitamin D Total (ng/mL)
Deficiency	< 10
Insufficiency	10 - 30
Sufficiency	30
Toxicity	>100

Paediatric reference intervals have not been established, but the American Association for Paediatrics (AAP) recommends a value of 20 ng/mL for healthy children.

Several population studies have identified widespread 25OH Vitamin D insufficiency ($>40\%$ of the population) in apparent healthy populations.

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About 70 percent of the United States population has inadequate levels of vitamin D. Groups that are at higher risk for inadequate levels of 25OH Vitamin D levels include individuals with limited sun exposure, the elderly, dark-skinned individuals and breast-feeding infants.

MEASUREMENT OF 25OH VITAMIN D

The two main types of methods are competitive immunoassay and those based on chromatographic separation followed by non-immunological direct detection (HPLC, LC-MS/MS). Lack of a reference standard for 25OH Vitamin D has, until recently, been a major issue resulting in poor between-method comparability. For immunoassays, specificity can be an issue especially in relation to the proportion of 25OH Vitamin D2 that is quantified whereas HPLC and LC-MS/MS methods are able to measure the two major vitamin D metabolites 25OH Vitamin D2 and 25OH Vitamin D3 independently. HPLC and LC-MS/MS require more expensive equipment and expert staff. In general precision of immunoassay, HPLC and LC-MS/MS are comparable and all have the required sensitivity to identify severe Vitamin D deficiency.

ANTIBODIES TO 25OH VITAMIN D

Polyclonal antibodies, from sheep or rabbit, have been widely used from the 90s, in RIA (RadioImmunoAssay), ELISA (Enzyme-Linked ImmunoSorbent Assay) and CLIA (ChemiLuminescence ImmunoAssay).

While polyclonal antibodies are simple and inexpensive to produce, they usually lack specificity and suffer from batch to batch variability. Monoclonal antibody production require high skills and is more expensive. However, a careful selection process ensures a superior specificity profile. Moreover, state of the art production techniques ensure a constant and renewable source of antibodies and all batches are identical.

In 2009, DIAsource Immunoassays patented Mouse Monoclonal Antibodies, based on a proprietary Vitamin D hapten, recognizing both 25OH Vitamin D3 and 25OH Vitamin D2. The patent was granted in Europe in 2013 and is still pending in the US. The patent covers any monoclonal antibody recognizing both 25OH Vitamin D3 and 25OH Vitamin D2.

These monoclonal antibodies have been successfully used in commercial RIA (RadioImmunoAssay), ELISA (Enzyme-Linked ImmunoSorbent Assay) and POCT (Point Of Care Test), by DIAsource Immunoassays and other companies.

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2. PRODUCT DATA SHEET

PRODUCT NAME	Monoclonal antibody anti 25OH Vitamin D Total
SPECIES	Mouse
CLONES	LMBP7012CB, Cat# 5319716 LMBP7013CB, Cat# 5319706
PRODUCT DESCRIPTION	Mouse monoclonal antibodies recognizing both 25OH Vitamin D3 and 25OH Vitamin D2

LMBP7012CB, Cat# 5319716

FORMAT	Purified, Liquid (PBS 0.1M, pH 7.4)
CONCENTRATION	>1mg/mL
PRESERVATIVE	NaN ₃ (0.05%)
PURIFICATION	Protein A affinity chromatography
ISOTYPE	IgG1 kappa
ISOELECTRIC POINT	7.35
IMMUNOGEN	Vitamin D derivative – BSA
MOUSE / FUSION PARTNERS	Balb/c (Charles River)/ NSO
SPECIFICITY	Recognizes native 25OH Vit D2 and Vit D3
STORAGE & SHELF LIFE	2 - 8°C / 10 years

LMBP7013CB, Cat# 5319706

FORMAT	Purified, Liquid (PBS 0.1M, pH 7.4)
CONCENTRATION	>1mg/mL
PRESERVATIVE	NaN ₃ (0.05%)
PURIFICATION	Protein A affinity chromatography
ISOTYPE	IgG1 kappa
ISOELECTRIC POINT	6.55
IMMUNOGEN	Vitamin D derivative – BSA
MOUSE / FUSION PARTNERS	Balb/c (Charles River)/ NSO
SPECIFICITY	Recognizes native 25OH Vit D2 and Vit D3
STORAGE & SHELF LIFE	2 - 8°C / 10 years

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Vit D ₂ ^b	1.3%	0.1%
24,25(OH) ₂ Vit D ₃ ^c	≥100%	≥100%
25,26(OH) ₂ Vit D ₃	≥100%	≥100%
3-epi-25OH Vit D ₃	0.1%	0.4%

^a Circulating concentrations are about 1‰ of 25OH Vitamin D concentrations.

^b Circulating concentrations are <5ng/mL.

^c The numbers provided have been obtained with spiked samples. Native samples containing various concentrations of 24,25(OH)₂ Vit D₃, as verified by LC-MS/MS, have not been biased in an ELISA assay.

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TYPICAL STANDARD CURVE

RIA (RadioImmunoAssay)	
25OH Vitamin D	cpm
0 ng/mL	21958
2 ng/mL	17916
6 ng/mL	14170
15 ng/mL	10710
55 ng/mL	4949
120 ng/mL	3161

LOD 0.4ng/mL RIA (RadioImmunoAssay)

LINEARITY Recovery on spiking 91 – 105 % 25OH Vitamin D3
96 – 101 % 25OH Vitamin D2
Recovery on dilution 100 – 123 %

SPECIFICITY

Compound	% Cross-reactivity ELISA	% Cross-reactivity RIA
25OH Vit D ₃	100%	100%
25OH Vit D ₂	83%	95%
1,25(OH) ₂ Vit D ₃ ^a	20%	4%
1,25(OH) ₂ Vit D ₂ ^a	1.9%	4%
Vit D ₃	2.9%	<0.3%
Vit D ₂	1.3%	<0.3%
24,25(OH) ₂ Vit D ₃	≥100%	≥100%

^a Circulating concentrations are about 1‰ of 25OH Vitamin D concentrations.

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4. PRODUCT ANALYSIS

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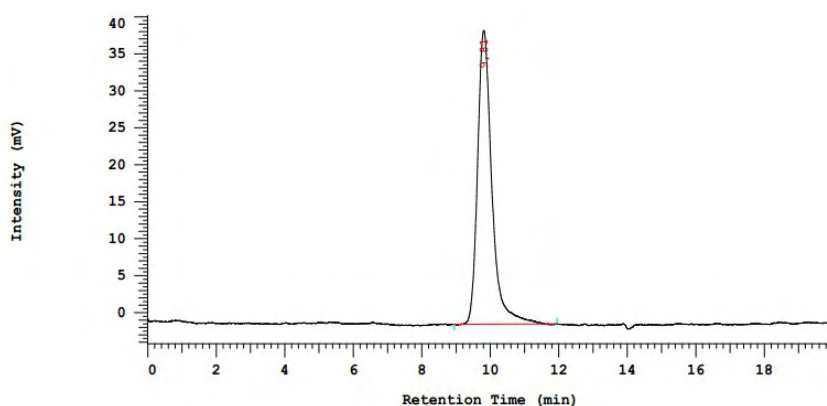
ANTIBODY CONCENTRATION UV 280nm (1mg/mL = 1.45 OD)

FUNCTIONAL TEST RIA & ELISA

SIZE EXCLUSION CHROMATOGRAPHY

Pre-column TSKgel SWXL Guardcolumn 6.0 mm x 4 cm
Column TSKgel G3000SWXL 7.8 mm x 30 cm
Eluent PBS 0.1M, 0.1M Na₂SO₄, 0.05% NaN₃, pH 6.7
Flow 0.9 mL/min
Concentration 2 mg/mL
Volume 10 µL
Wavelength 280 nm

Last batch produced B14A4P1



No.	RT	Area	Conc 1	BC
1	9,81	1144465	100,000	MC
		1144465	100,000	

Overlap of 3 consecutive batches produced (B9F1P1, B9I2P1, B12C3P1)

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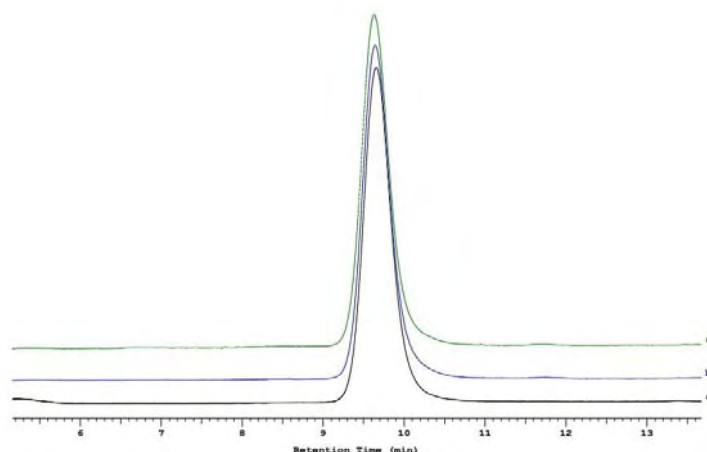
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LMBP7013CB, Cat# 5319706

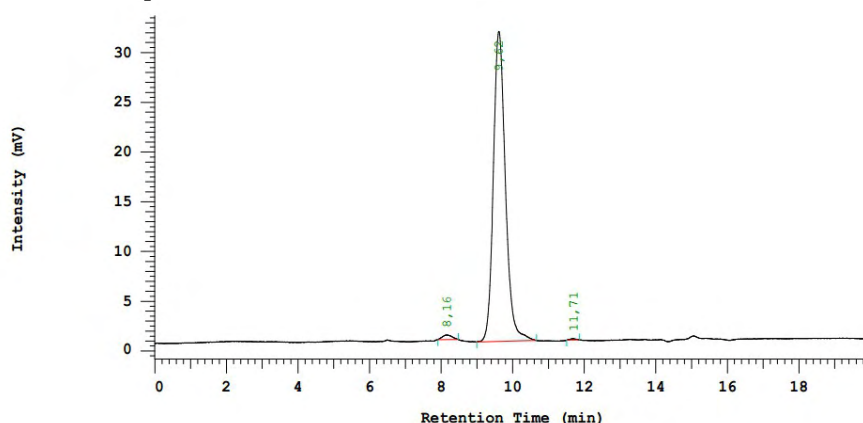
ANTIBODY CONCENTRATION UV 280nm (1mg/mL = 1.45 OD)

FUNCTIONAL TEST RIA

SIZE EXCLUSION CHROMATOGRAPHY

Pre-column TSKgel SWXL Guardcolumn 6.0 mm x 4 cm
 Column TSKgel G3000SWXL 7.8 mm x 30 cm
 Eluent PBS 0.1M, 0.1M Na₂SO₄, 0.05% NaN₃, pH 6.7
 Flow 0.9 mL/min
 Concentration 2 mg/mL
 Volume 10 µL
 Wavelength 280 nm

Last batch produced B10B4



No.	RT	Area	Conc 1	BC
1	8,16	8686	1,181	BB
2	9,62	725601	98,623	BB
3	11,71	1442	0,196	BB
		735729	100,000	

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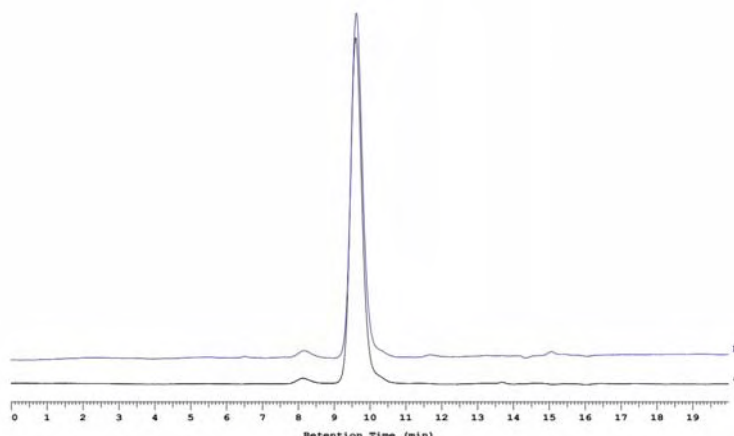
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Overlap of 2 consecutive batches produced (B9F1P1P2, B10B4)



5. VITAMIN D STARTER KIT

Over the years DIAsource ImmunoAssay has built extensive experience in Vitamin D immunoassay development and Vitamin D chemistry.

DIAsource ImmunoAssays offers all components to develop your own Vitamin D assay.

ANTIBODIES

Based on a proprietary Vitamin D hapten, we have developed a line of mouse monoclonal antibodies directed towards 25-hydroxyvitamin D2 and D3. These monoclonal antibodies have been successfully used in commercial RIA (RadioImmunoAssay), ELISA (Enzyme-Linked ImmunoSorbent Assay) and POCT (Point Of Care Test), by DIAsource Immunoassays and other companies.

ANTIGENS

We have developed a specific collection of Vitamin D derivatives that go along with our monoclonal antibodies. Depending on your specific application, you may want to use our carboxylic acid, amine, biotin or BSA derivatives. These Vitamin D derivatives are produced by our highly skilled synthetic chemists in state of the art organic chemistry facilities.

Furthermore, we offer services tailored to your specific requirements.

DISPLACEMENT SOLUTION

Displacing 25-hydroxyvitamin D from its binding proteins (VDBP) is always a big challenge in Vitamin assay development. DIAsource offers a unique displacement solution that features the All-in-One (AIO) technology.

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WO2011051348, EP2316854, US2011097733

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