

Technical Note

## Development of a 25OH Vitamin D ELISA assay based on DIAsource raw materials and key components

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## 1. INTRODUCTION

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

DIAsource ImmunoAssays offers all the raw materials and key components required to develop your own 25OH Vitamin D assay.

This document describes the essential steps to the development of a 25OH Vitamin D ELISA assay using the DIAsource 25OH Vitamin D monoclonal antibodies, antigens, release solution and key assay components.

## 2. MATERIAL

### 2.1. Key raw materials

It is important to emphasize that the DIAsource anti-25OH Vitamin D antibodies have been specifically designed to recognize both the D2 & D3 forms of 25OH Vitamin D and only work in combination with the DIAsource total Vitamin D derivatives. The classical 25OH Vitamin D conjugates functionalized on position 3 are not recognized by the DIAsource antibodies.

Cat. #	Description	Format	Comment
5319716	Mouse monoclonal antibody against 25OH Vitamin D2 & D3	Purified, liquid (Phosphate buffer)	Patented (EP2316854, US20110097733), additional clones available
5019701	Total Vitamin D derivative – BSA conjugate	Purified, liquid	Specific for DIAsource antibodies
5019708	Total Vitamin D derivative – biotin conjugate	Purified, dry	Specific for DIAsource antibodies
4219713	25OH Vitamin D release solution	Liquid	Works instantaneously and at neutral pH

### 2.2. Other specific components

In addition to the key raw materials listed above, DIAsource can provide specific 25OH Vitamin D calibrators, controls, coated microtiterplates and concentrated conjugate.

Calibrators and controls can be sourced from DIAsource or can be developed by your company.

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Coated microtiterplates and concentrated conjugate can be sourced from DIAsource or can be developed from respectively the above-described monoclonal antibody (Cat. #5319716) and total Vitamin D derivative – biotin conjugate (Cat. #5019708).

Cat. #	Description	Format	Comment
4219712	25OH Vitamin D Calibrator 0	Lyophilized	Available in unit and bulk quantities
4219719	25OH Vitamin D Calibrators 1-5	Lyophilized	Available in unit and bulk quantities
4219708	Microtiterplate coated with anti-25OH Vitamin D2 & D3	96 wells	Breakable wells
4119703	Concentrated conjugate	Liquid	Total Vitamin D derivative – biotin conjugate (Cat. #5019708) in a stabilizing buffer

### 3. DEVELOPMENT OF A 25OH VITAMIN D ELISA ASSAY

#### 3.1. Antibody coating

The mouse monoclonal antibody against 25OH Vitamin D2 & D3 (Cat. #5319716) is diluted into the coating buffer containing an appropriate blocking protein. The concentration of the antibody, the nature and concentration of the coating buffer and blocking protein need to be optimized.

The obtained coating solution is dispensed into ELISA microplates (e.g. Nunc Maxisorb or Costar High Bind) and incubated for 24-72h at 2-8°C. Microplates are washed, dried down and packaged in aluminum pouches with desiccant.

Alternatively, the mouse monoclonal antibody can be coated into microplates which have been pre-coated with an anti-mouse antibody (e.g. rabbit anti-mouse or goat anti-mouse).

#### 3.2. ELISA incubation without 25OH Vitamin D

To the coated microplate, add the total Vitamin D derivative – biotin conjugate (Cat. #5019708) diluted in an appropriate buffer containing 1-5g/L protein. Incubate at 18-37°C for 30-120', wash and add diluted Streptavidin-HRP. Incubate at 18-37°C for 15-60' and wash.

Add diluted TMB, incubate at 18-37°C for 15-30' then add Stop (HCl or H<sub>2</sub>SO<sub>4</sub>) and read at appropriate wavelength.

Alternatively, DIAsource coated microplates (Cat. #4219708) and DIAsource concentrated conjugate (Cat. #4119703) can also be used.

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Alternatively, diluted Streptavidin-HRP can be added directly after the total Vitamin D derivative – biotin conjugate.

Alternatively, diluted Streptavidin-HRP can be pre-mixed with the total Vitamin D derivative – biotin conjugate and the mixture is then added to the microplates.

### 3.3. ELISA incubation with 25OH Vitamin D in buffer

To the coated microplate, add the total Vitamin D derivative – biotin conjugate (Cat. #5019708) diluted in an appropriate buffer containing 1-5g/L protein and add 25OH Vitamin D3 diluted in an appropriate buffer (dilute 1% of 25OH Vitamin D3 in ethanol to 99% of an aqueous buffer containing 1-5g/L protein, final 25OH Vitamin D3 concentration from 0 to 150ng/mL). Incubate at 18-37°C for 30-120', wash and add diluted Streptavidin-HRP. Incubate at 18-37°C for 15-60' and wash.

Add diluted TMB, incubate at 18-37°C for 15-30' then add Stop (HCl or H<sub>2</sub>SO<sub>4</sub>) and read at appropriate wavelength.

Test the alternatives proposed in 3.2.

Alternatively, to the coated microplate, add 25OH Vitamin D3 diluted in an appropriate buffer (dilute 1% of 25OH Vitamin D3 in ethanol to 99% of an aqueous buffer containing 1-5g/L protein, final 25OH Vitamin D3 concentration from 0 to 150ng/mL). Incubate at 18-37°C for 30-120', wash and add the total Vitamin D derivative – biotin conjugate (Cat. #5019708) diluted in an appropriate buffer. Incubate at 18-37°C for 30-120', wash and add diluted Streptavidin-HRP. Incubate at 18-37°C for 15-60' and wash.

Add diluted TMB, incubate at 18-37°C for 15-30' then add Stop (HCl or H<sub>2</sub>SO<sub>4</sub>) and read at appropriate wavelength.

Test the alternatives proposed in 3.2.

### 3.4. ELISA incubation with 25OH Vitamin D in human samples

In human samples 25OH Vitamin D is tightly bound to binding proteins. A release reagent is required to release the 25OH Vitamin D from its binding proteins. The release solution provided by DIAsource (Cat. #4219713) can work in presence of the antibodies, instantaneously and at neutral pH.

To the coated microplate, add the human sample (typically serum but plasma can also be used) and add the 25OH Vitamin D release solution (Cat. #4219713). Add the total Vitamin D derivative – biotin conjugate (Cat. #5019708) diluted in an appropriate buffer containing 1-5g/L protein and incubate at 18-37°C for 30-120', wash and add diluted Streptavidin-HRP. Incubate at 18-37°C for 15-60' and wash.

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Add diluted TMB, incubate at 18-37°C for 15-30' then add Stop (HCl or H<sub>2</sub>SO<sub>4</sub>) and read at appropriate wavelength.

Test the alternatives proposed in 3.2.

Alternatively to the coated microplate, add the human sample and add the 25OH Vitamin D release solution (Cat. #4219713). Incubate at 18-37°C for 30-120' and wash. Add the total Vitamin D derivative – biotin conjugate (Cat. #5019708) diluted in an appropriate buffer containing 1-5g/L protein and incubate at 18-37°C for 30-120', wash and add diluted Streptavidin-HRP. Incubate at 18-37°C for 15-60' and wash.

Add diluted TMB, incubate at 18-37°C for 15-30' then add Stop (HCl or H<sub>2</sub>SO<sub>4</sub>) and read at appropriate wavelength.

Test the alternatives proposed in 3.2.

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## Ordering Information

Description	Article code	Format
25OH Vitamin D Total ELISA kit	KAP1971	ELISA kit
Mouse monoclonal antibody against 25OH Vitamin D2 & D3	5319716	Liquid
Total Vitamin D derivative – BSA conjugate	5019701	Liquid
Total Vitamin D derivative – biotin conjugate	5019708	Dry
25OH Vitamin D release solution	4219713	Liquid
25OH Vitamin D Calibrator 0	4219712	Lyophilized
25OH Vitamin D Calibrators 1-5	4219719	Lyophilized
Microtiterplate coated with anti-25OH Vitamin D2 & D3	4219708	96 wells
Concentrated conjugate	4119703	Liquid



### Headquarter

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