Tribioscience

7-Dehydrocholesterol (7-DHC) Fast ELISA Catalog Number: TBS32128

For the quantitative determination of 7-DHC concentrations in biological samples

Introduction

7-Dehydrocholesterol (7-DHC) is a cholesterol precursor in the serum and is photochemically converted to vitamin D3 in the skin. Its functioning serves as a provitamin-D3 to vitamin D in the body upon exposure to ultraviolet B (UVB) radiation from sunlight. A deficiency in 7-DHC can lead to various health issues, such as increased risk of osteoporosis, cancer and higher susceptibility to infections. The Tribo 7-DHC ELISA Kit can **quickly, sensitively, and accurately** determine the presence of 7-DHC in CSF, urine, serum and other biological samples.

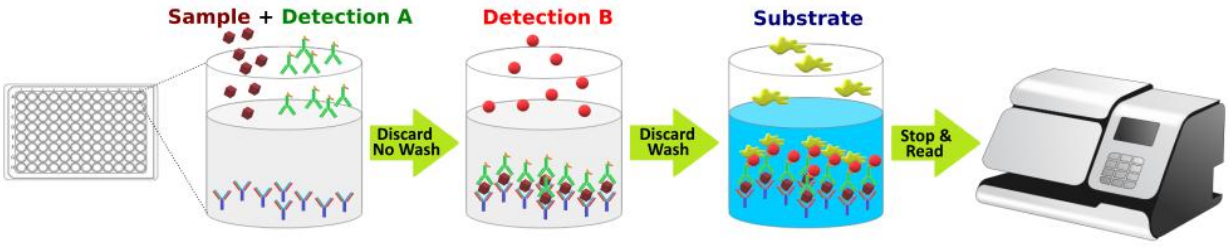
Intended Use

The Tribo 7-DHC ELISA Kit utilizes competitive ELISA for the quantitative and qualitative analysis of 7-DHC in biological samples. The limit of detection (LOD) of 7-DHC in ELISA Kit is 0.2ng/ml.

Assay Principle

Tribo 7-DHC ELISA Kit is a competitive enzyme-labeled immunoassay (Fig. 1). The 96- well microtiter plate is pre-coated with an anti-7-DHC antibody. During the assay, 7-DHC standard solution or samples are added to each well, followed by adding biotin conjugated competitor (Detection A), which compete with 7-DHC in standard or sample for binding to the antibody coated in the plate during the incubation. Then, discard the liquid, add Streptavidin-HRP (Detection B) and incubate. After plate wash, an ultra-sensitive HRP substrate is added to the wells leading to a colored product only in the presence of HRP, and optical density is inversely related to 7-DHC concentrations in the samples. The accurate concentration of 7-DHC can then be determined by interpolation using the standard curve constructed in the same run. The main feature is that the kit uses our novel proprietary approaches to combine samples and detections into a one-step instead of the complicated traditional methods. It makes the assay simple, easy, accurate and fast.

Fig. 1. Simple Procedures



Kit Contents

PART	PART#	48 Strip-well ELISA	96 Strip-well ELISA	DESCRIPTION	Storage
Pre-coated Microplate	TBS32128A	48 Strip-well plate	96 Strip-well plate	48 or 96 Strip-well microplate coated with an antibody specific to 7-DHC.	4°C
7-DHC Standard	TBS32128B	300 µL	600 µL	Total 7-DHC (200 ng/mL).	-20°C
Detection A (100x)	TBS32128C	11 µL	22 µL	Biotin-labeled Competitor (100x).	-20°C
Detection B (100x)	TBS32128D	55 µL	110 µL	Streptavidin-HRP (100x).	-20°C
Assay Diluent	TBS32128E	8 mL	13 mL	Assy Diluent.	4°C
Wash Buffer (10x)	TBS3000W	12 mL	12 mL	Concentrated Buffer (10x).	4°C
TMB Substrate	TBS3000T	6 mL	12 mL	Ultra-sensitive TMB substrate.	4°C
Stop Solution	TBS3000S	3 mL	6 mL	2 N sulfuric acid.	4°C

The kit contains sufficient materials to run an ELISA on a half or one 96 well plate.

Safety Instructions

To receive complete safety information on this product, contact Tribioscience, Inc. and request Material Safety Data Sheets. Stop solution is 2N sulfuric acid. Handle with care.

Sample preparation for ELISA assay

The sample to be tested should be collected according to accepted sampling techniques.

ELISA Procedures

Bring all reagents to room temperature before use.

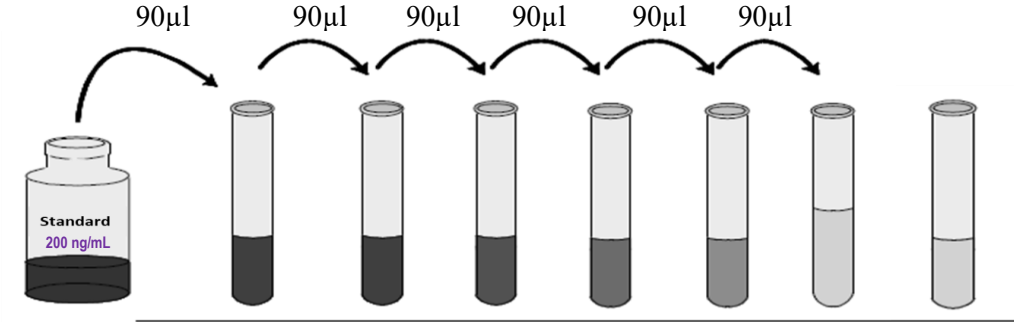
Wash Buffer: Add 12 mL of Wash Buffer Concentrate (10x) to 108 mL of deionized distilled water to prepare 120 mL of Wash Buffer (*If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved).*

Detection A and B preparation: Dilute 100x detection A and detection B with assay buffer before using it.

7-DHC Standard Preparation: The provided Standard is used as the highest concentration of 200 ng/mL in Std1. Label test tubes as #2 through #8. Pipet 180 µL of 1x Assay Diluent into tube #2 to #8 **as diagram below.**

1. Add 90 µL of the 7-DHC Standard solution (200ng/mL as Std1 provided in the Kit to tube #2 and mix.
2. Make 3x serial dilutions of the standard using the Tube#2 (66 ng/mL standard solution) from Tube #3 through #7 with sequential transfer of 90 µL to the next concentration. Mix each tube thoroughly and gently before the next transfer. The standard concentration in tube 1 through 7 will be 200, 66, 22, 7.3, 2.4, 0.8, and 0.27 ng/mL. Tube# 8 is Standard 0.

Fig.2 Diagram for 7-DHC standard preparation



	Std 1	Std2	Std3	Std4	Std5	Std6	Std7	Std8
Assay Buffer (µL)	0	180	180	180	180	180	180	180
Addition	0	Stock	Std1	Std2	Std3	Std4	Std5	
Addition Vol. (µL)	0	90	90	90	90	90	90	0
Final Conc (ng/ml)	200	66	22	7.3	2.4	0.8	0.27	0

Assay Procedures:

1. Add 80 µL of standards, samples, or control into the appropriate wells.
2. Add 20 µL of **Detection A** to the above of each well immediately, thoroughly and gently mix. Cover with the adhesive or plate lid. Incubate at **37°C for 60 minutes**.
3. Aspirate each well liquid and add 100µL of **Detection B** to each well. Incubate at **37°C for 30 minutes**.
4. Aspirate each well liquid and wash 4 times by filling each well with 200 µL Wash Buffer, incubate at RT for 2-3min for each time wash (*Complete removal of liquid at each step is essential to good performance*). After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **TMB Substrate** to each well. Incubate **at 37°C for 10-20 minutes** (*Protect from light*). The color becomes blue.
6. Add 50 µL of **Stop Solution** to each well. The color in the well should change from blue to yellow (gently tap the plate to ensure thorough mixing).
7. Read at 450 nm.

Quantitative Calculation of 7-DHC Concentration

a) Calculate B/B0

Dividing average absorbance of each standard and sample (B) by absorbance of standard 0 (the standard with 0 ng/mL of 7-DHC, B0) to obtain percentage absorbance.

percentage absorbance (%) = B/B0 x 100

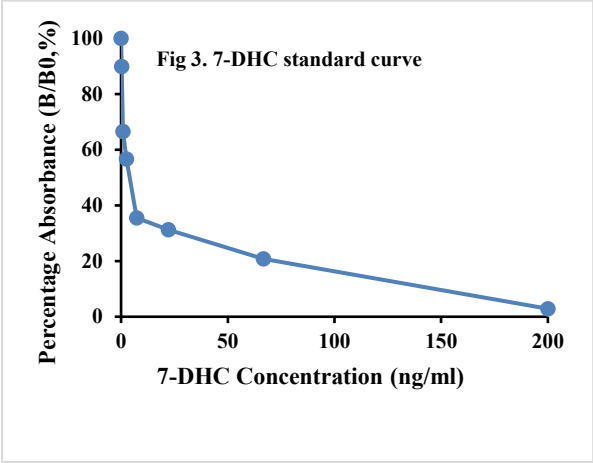
B: Average absorbance of a standard or sample

B0: Average absorbance of 0 ng/mL standard

b) A standard curve is obtained by graphing the percentage absorbance of standards (Y axis) versus their corresponding concentration (X axis) (example as below), and sample concentration can be read from this standard curve. Alternatively, 7-DHC concentration in the samples can be calculated with regression equations correlating percentage absorbance to 7-DHC concentration.

Typical Data

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed. **Fig. 3** is an example of typical Data.



Technical Assistance

For ordering or technical assistance regarding this kit, or for additional information about TribioScience products, please email info@tribioscience.com or call (408) 498-0197.

Relative Products

Human p-Tau-217 ELISA (TBS3293)
Human p-Tau-181 ELISA (TBS3294)
Human Total Tau ELISA (TBS3295)
Human p-Tau-231 ELISA (TBS3296)
Human AD7c NTP (TBS3297)
Human Amyloid ß40 ELISA (TBS3298)
Human NF-L ELISA (TBS32101)
Human Total Amyloid ß ELISA (TBS32104)
Human UCHL1/PGP9.5 ELISA (TBS32107)
Human Gamma H2AX ELISA (TBS3265)
Human H2AX ELISA (TBS3266)
Human IL-4 ELISA (TBS3221)
Human IL-4 ELISA (TBS3221)
Human IL-6 ELISA (TBS3223)
Human IL-7 ELISA (TBS3224)
Human IL-8 ELISA (TBS3225)
Human IL-10 ELISA (TBS3226)
Human IL-13 ELISA (TBS3227)
Human IL-17 ELISA (TBS3228)
Human IFN-gamma ELISA (TBS3230)
Human TGF- ß1 ELISA (TBS3232)
Human GM-CSF ELISA (TBS3233)
Human MIP-1α ELISA (TBS3234)
Protein Cell Lysis Buffer (TBS5001)

For research use only.