Vitamin A Food ELISA Kit

Cat.No: DEIA-CL016
Lot. No. (See product label)

Size

96T

Intended use

CD provides materials for the quantitative measurement of vitamin-A in dairy products. This assay is intended for in vitro quantification only.

General Description

Dairy milk is fortified with vitamins A & D, as milk has become the major source of these vitamins for human beings. Regulatory agencies have set standards specifying the amount of vitamins A and D to be added to milk products. The methodology has been designed to extract the vitamins from milk fat, and to directly quantify the amount of vitamins in the ELISA based assay. Other methods that can detect vitamins in dairy milk are time consuming and require specialized laboratory equipment and trained personnel.
CD provides materials for the quantitative measurement of vitamin-A in dairy products. This assay is intended for in vitro quantification only.

Principle Of The Test

The vitamin A ELISA test is based on the principle of a sandwich enzyme-linked immunosorbent assay. The assay system utilises immobilization of a monoclonal antibody directed against a distinct antigenic determinant on the vitamin-A molecule (retinol) on a solid phase. A second Anti-vitamin-A monoclonal antibody conjugated to horseradish peroxidase (HRP) is used as the detecting antibody in the assay. The test sample is allowed to react sequentially with the two antibodies, resulting in the vitamin-A molecules being sandwiched between the solid phase and enzyme-linked antibody.
After the incubation, the wells are washed with distilled water to remove all unbound labelled antibodies. TMB solution is added as a substrate, resulting in the development of a blue color. The reaction is stopped with the addition of 0.2M H2SO4, changing the color to yellow. The concentration of vitamin A is directly proportional to the color intensity of the test sample. Absorbence is measured spectrophotometrically using a plate reader at 450 nm.

Reagents And Materials Provided

1. **SORB** Anti-Vitamin-A antibody coated wells: 96 wells with Anti-Vitamin-A Mab immobilized on the wells.
2. **CONJ ENZ** Anti-Vitamin-A antibody conjugate with HRP: one (1) vial containing0.1 mL ofconcentrated Anti-Vitamin-A conjugated withHRP, in a stabilizer solution.
3. **CAL 1-5** Vitamin-A Standard: Standards prepared in hexane: 0.0, 0.055, 0.11, 0.22, 0.45 IU/mL. Content is 0.5 mL per vial.
4. **CONTROL 1** Control 0.3 IU/mL: 0.5 mL per vial.
5. **CONTROL 2** Control 0.1 IU/mL: 0.5 mL per vial.
6. **BUF** Reaction Buffer one (1) vial containing 13 mL of protein based (milk)buffer with thimerosal as preservative.

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7. **SUBS TMB** Enzyme substrate: one (1) vial containing 11 mL of TMB solution.
8. **CONJ DIL** Conjugate Diluent: one (1) vial containing 11 mL of glycine based Buffer.
9. **H2SO4** Stopping solution: one (1) vial containing 12 mL of 0.2 M sulfuric acid.
10. **STAB** Stabilizer: one (1) vial containing 0.15 mL of stabilizer.

Materials Required But Not Supplied

1. Precision pipettes with disposable tips
2. 8 channel pipette (100-200 µL) with disposable tips
3. Plate shaker set at 180 ± 10 rpm
4. Microplate reader with filter at 450 nm
5. Microplate washer
6. Centrifuge
7. Vortex Mixer
8. Deionized or distilled water
9. Absorbent paper
10. 95% ethanol (denatured)
11. Potassium hydroxide (KOH) pellets
12. Hexane
13. 10 mL screw capped glass tubes
14. 1 or 2 mL screw capped amber glass vials

Storage

2-8°C

Specimen Collection And Preparation

Bring fluid milk container to room temperature. Rotate slowly at least 10 times without foaming. Extractions are slightly different based on the percentage of milk fat as described below and summarized in Table I

A. Milk with 3.25 %M.F., 2%M.F., and 1%M.F.
1. Label 10 mL screw capped glass tubes and pipette 1 mL of milk in corresponding tube. Pipette 15 µL of ethanol into each tube, Do not cap the tubes. Shake for 20 seconds in the dark.
2. Add 0.60 g of KOH into milk with 3.25% and 2% milk fat, and 0.50g into 1% milk fat. Gently mix for 2 minutes in the dark. Cap the tubes and incubate at room temperature for 4 minutes in the dark. Shake vigorously for 2 minutes. Repeat 4 minute incubation and 2 minute vigorous shaking 2 more times (totals 12 minute incubation and 6 minute shaking).
3. Pipette 2 mL of hexane into above solution. Cap and shake vigorously for another 2 min in the dark.
4. Centrifuge each tube at room temperature for 5 minutes at 2400 rcf. After centrifugation, handle tubes carefully. The upper organic phase must be perfectly clear and well separated.
5. Label 1 or 2 mL screw capped amber coloured glass vials and add 300 µL of hexane into each vial for dilution of vitamin-A extract. Transfer 100 µL of vitamin-A extract in corresponding amber coloured glass vials (100 µL of organic phase + 300 µL of hexane). The amber coloured glass vials, which contain 8-fold diluted vitamin A extract, must be capped well and should be assayed immediately. If necessary dilute the extract with more hexane.

B. Skim Milk
1. Label 10 mL screw capped glass tubes and pipette 1 mL of milk into corresponding tube. Pipette 1mL of distilled water into one tube as a water control. Pipette 15 µL of ethanol into each tube and gently mix for 20 seconds in the dark.

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2. Add 0.45 g of KOH into each tube and gently mix for 2 minutes in the dark. Add 5μL of stabilizer STAB. Cap and vortex tubes for 10 seconds. Incubate at room temperature for 4 minutes in the dark. Shake vigorously for 2 minutes. Repeat 4 minute incubation and 2 minute vigorous shaking 2 more times (totals 12 minute incubation and 6 minute shaking).
2. Pipette 2 mL of hexane into above solutions. Cap and shake vigorously for another 2 minutes in the dark.
3. Centrifuge each tube at room temperature for 5 minutes at 2500 rpm.
4. Label 1 or 2 mL screw capped amber coloured glass vials and add 300 μL of hexane into each vial for dilution of extract. After centrifugation, handle tubes carefuly. The upper organic phase must be perfectly clear and well separated. Transfer 100 μL of upper extract in corresponding amber coloured glass vials (100 μL of organic phase + 300 μL of hexane). The amber coloured glass vials, which contain 8-fold diluted extract, must be capped very well and shoul be assayed immediately. If necessary, dilute the extract with more hexane.

Table I

	Steps	3.25 & 2%M.F.	1% M.F.	Skim milk	Distilled water	Conditions
	Fluid Milk	1 mL	1 mL	1 mL	1 mL	Run water control only for skim milk.
Saponification and extraction	Ethanol 95%	15 μL	15 μL	15 μL	15 μL	Gently mix for 20 seconds.
	KOH (g)	0.6	0.5	0.45	0.45	Gently mix for 2 minutes in the dark.
	Stabilizer	-	-	5 μL	5 μL	Vortex for 10sec. (skim milk & water control only).
	Incubate 4 minute, and shake vigorously for 2 minute in the dark. Repeat 4 minute incubation and 2 minutes vigorous shaking two more times (totals 12 min. incubation and 6 min. shaking)					
	Hexane	2 mL	2 mL	2 mL	2 mL	Shake vigorously for 2 minutes in the dark and centrifuge at 2400 rcf for 5 minutes.
Extract dil. with hexane	Upper organic phase	100 μL	100 μL	100 μL	100 μL	The diluted extract in screw capped amber coloured glass vial should be assayed immediately.
	Hexane	300 μL	300 μL	300 μL	300 μL	

Reagent Preparation

1. Bring all reagents to room temperature before use (22°C ± 2°C), except enzyme conjugate concentrate CONJ ENZ (EC) which should be at kept 2°C - 8°C
2. Enzyme conjugate concentrate (EC) should be diluted as indicated on the bottle (label) with conjugate diluent (CD) according to the number of wells used. Mix the enzyme conjugate concentrate by pipetting 2-3 times with a pipette tip before diluting with diluent. Add required amount of enzyme conjugate concentrate to the conjugate diluent and mix thoroughly before use. Diluted conjugate can not be stored and should be prepared fresh for each assay run.

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Handling notes:
Do not mix materials from different kit lots.
Bring all reagents to room temperature before using except Anti-Vitamin-A antibody conjugate with HRP.
Use a clean disposable pipette tip for addition of each different sample and reagent to avoid cross-contamination.
Use only glass vials for the extraction of vitamins.
Prepare a standard curve for each run. Do not use data from previous runs.
Cap all Vitamin-A calibrators and Vitamin-A extracted specimens immediately after loading onto ELISA plate. This will allow the reference calibrators and extracts to be used more than once if desired.
Load all extracted specimens and reference calibrators within 5 minutes and accurately onto the ELISA strips to limit variations in evaporation time between the first and last well loaded.
All hexane steps should be performed under the fume hood.

Assay Procedure

Refer to the assay procedure, Table II.
Standards, specimens and controls shoud be assayed in duplicate.
Secure the desired number of coated wells SORB in the holder.
1. Pipette 10 µL of calibrators CAL 1-5, controls CONTROL 1 , CONTROL 2 and diluted extracted specimens into the corresponding wells.
2. Shake the wells 6 minutes on a plate shaker (180 ± 10 rpm) at room temperature (22 ± 2°C) to evaporate hexane
3. Pipette 120 µL of Reaction Buffer BUF into each well. Mix gently for 30 seconds. Place opaque lid or adhesive cover over the strips.
4. Incubate for 30 minutes in the dark on the plate shaker (180 ± 10 rpm) at room temperature (22 ± 2°C).
5. Wash four times with distilled water using Microplate washer. Manual washing may also be used: with wash bottle or using multi-channel pipette, add 380 µl of distilled water in each well in each wash cycle. Care should be taken to avoid spillage of distilled water into adjacent wells. After the wash, completely decant the water by tapping the plate against absorbing paper until no trace of water is visible on the paper.
6. Mix the freshly diluted Anti-Vitamin-A conjugate-HRP. Pipette 100 µL of freshly diluted Anti-Vitamin-A conjugate-HRP (CONJ ENZ) in each well. Mix gently for 20 seconds. Place opaque lid or adhesive cover over the strips.
7. Incubate for 20 minutes in the dark at room temperature (22 ± 2°C).
8. Wash 6 times (refer to step no. 5).
9. Pipette 100 µL of TMB SUBS TMB (Substrate) into each well. Gently mix for 10 seconds.
10. Incubate 10 minutes in the dark at room temperature (22 ± 2°C).
11. Add 100 µL of the stopping solution H_2SO_4. Gently mix for 10 seconds.
12. Measure the absorbance at 450 nm using a microplate reader.

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TABLE II

Wells	Identification	Assay Volumer	Evaporate	Reaction Buffer			Dil. Conjugate			Substrate		Stop. Sol.	
A_1,A_2	0 IU/mL	10 µL		120 µL	INCUBATE	DECANT & WASH	100 µL	INCUBATE	WASH	100 µL	INCUBATE	100 µL	READ AT 450 nm
B_1,B_2	0.055 IU/mL												
C_1,C_2	0.11 IU/mL												
D_1,D_2	0.22 IU/mL												
E_1,E_2	0.45 IU/mL												
F_1,F_2	Control 1												
G_1,G_2	Control 2												
H_1,H_2	Sample extract		1		2	3		4	5		6		
etc...	etc...												

NOTE: READ THE ABSORBANCES IMMEDIATELY AFTER COMPLETING THE ASSAY.

Quality Control

Good laboratory practice requires that quality control specimens be run with each calibration curve to check the assay performance.

Calculation

The standard curve is used to determine the amount of Vitamin A in unknown sample. The standard curve is generated by plotting the average of O.D. (450 nm) obtained for each of the standard concentrations on the vertical (y) axis versus the corresponding standard concentrations on the horizontal (X) axis.
Examine data for acceptance criteria with quality control guidelines.
- For unknown samples, multiply the values by a factor of 8 as per the stipulated assay conditions.
- 3.33 I.U. of Vitamin A = 1 µg

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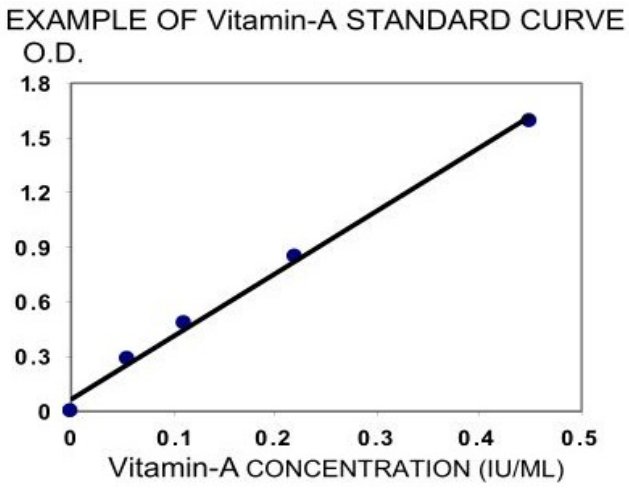

Performance Characteristics

Sensitivity: The range for this assay under the specified conditions is from 0.055 I.U./mL to 0.45 I.U./mL.
Precision & reproducibility: The relative standard deviation for interassay and intrassay was determined to be 2.4% and 2% respectively.
Cross Reactivity and Specificity: The kit did not exihibit any cross reactivity with cholesterol and is specific for Vitamin A.
Standard curve Linearity: Linearity was determined to be 0.996 (Average of six independent assays) with %RSD of 1.5%.

Precautions

1. All materials in this kit should be used only for in vitro quantitative tests not involving internal or external administration of the material to humans or animals.
2. Respect laboratory quality controls rules.
3. Reagents are matched in each kit, and, therefore, reagents from different lot numbers should not be mixed.
4. This kit should not be used after the expiration date.
5. Optimal results will be obtained by strict adherence to this protocol.
6. The stopping solution contains sulfuric acid. This solution should be handled with caution, avoiding skin contact.
7. KOH pellets should be handled with caution, avoiding skin contact.
8. Work all hexane steps should be performed under the hood.
9. Prior to assay, Bring all reagents to ambient temperature by allowing them to stand at room temperature (22 ± 2°C). Gently mix all reagents.

Limitations

a) Reliable and reproducible results will be obtained when the assay procedure is carried out with strict adherence to the procedure described within this package insert and good laboratory practice.
b) A maximal total pipetting time of 5 minutes for calibrators, controls and specimens is suggested.

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