FOR RESEARCH USE ONLY!

03/21

Filaggrin (Human) ELISA Kit

(Catalog # E4970-100, 96 assays, Store at 4ºC)

I. Introduction:

Filaggrin (FLG) is a critical structural protein in the stratum corneum of the skin epidermis. The protein is synthesized as a precursor (Profilaggrin) and is localized in keratohyalin granules in the stratum granulosum. During terminal differentiation of epidermal cells, Profilaggrin undergoes proteolysis to yield monomeric filaggrin units. Filaggrin regulates epidermal homeostasis. It also maintains skin barrier function. Additionally, it promotes the aggregation of keratin intermediate filaments to form a keratin network which contributes to the physical strength of the skin. Various proteases hydrolyze Filaggrin to yield amino acids and amino acid derivatives that function as natural moisturizing factors in the stratum corneum. Mutations in Filaggrin result in skin disorders such as atopic dermatitis and eczema. Autoantibodies in rheumatoid arthritis (RA) recognize citrullinated residues in Filaggrin. Hence antibodies to Filaggrin are used as markers to detect RA. BioVision's Filaggrin (Human) ELISA kit is used to detect Filaggrin in human serum, plasma, and other biological fluids. The kit is based on the Sandwich principle. Test samples, Standards, and Biotinylated Detection antibody are added to the wells pre-coated with capture antibody and then washed with Wash Buffer. The HRP-Streptavidin is added and any unattached conjugates are washed off by Wash Buffer. The HRP enzymatic reaction is detected by the addition of TMB-substrate. Finally, the reaction is terminated with an acidic stop solution. The color developed is directly proportional to the concentration of Filaggrin in the sample or standard.

II. Features and Benefits:

- Detection range: 0.313 – 20 ng/ml
- Sensitivity: 0.188 ng/ml
- Assay Precision: Intra-Assay CV < 8% and Inter-Assay CV < 10%
- Recovery range: 85 - 105% for normal human serum and plasma samples
- This Sandwich ELISA is highly sensitive and highly specific for the detection of FLG in human samples. There is no significant cross-reactivity or interference between FLG and analogues

III. Sample Type:

Human Serum, Plasma, Tissue lysates and other biological fluids

IV. Kit Contents:

Components	E4970-100	Part Number	Storage Temp.
Micro ELISA plate	8 x 12 Strips	E4970-100-1	-20°C
Standard (Lyophilized) (12 ng)	2 vials	E4970-100-2	-20°C
Sample/Standard Dilution Buffer	20 ml	E4970-100-3	4°C
Biotin-labeled Antibody	120 µl	E4970-100-4	4°C (Avoid light)
Antibody Dilution Buffer	10 ml	E4970-100-5	4°C
HRP-Streptavidin Conjugate (SABC)	120 µl	E4970-100-6	4°C (Avoid light)
SABC Dilution Buffer	10 ml	E4970-100-7	4°C
TMB Substrate Solution	10 ml	E4970-100-8	4°C (Avoid light)
Stop Solution	10 ml	E4970-100-9	4°C
Wash Buffer (25X)	30 ml	E4970-100-10	4°C
Plate Sealers	5	E4970-100-11	4°C

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 37°C incubator
- Precision pipettes with disposable tips
- Distilled or deionized water
- Clean eppendorf tubes for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit can be stored at 4°C for up to 6 months from the date of shipment.

VII. Reagent and Sample Preparation:

Note: Prepare reagents within 30 minutes before the experiment

Before using the kit, spin tubes and bring down all components to the bottom of tubes

1. **Biotin-labeled Antibody working solution:** Prepare this working stock 1 hour prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1 – 0.2 ml to the total volume. Dilute the Biotin-labeled antibody with Antibody Dilution Buffer at 1:100. Mix thoroughly.
2. **HRP-Streptavidin Conjugate (SABC):** Prepare this working stock 30 minutes prior to the start of the experiment. Calculate the total volume of the working solution: 0.1 ml/well x quantity of wells. Add 0.1 – 0.2 ml to the total volume. Dilute the SABC with SABC Dilution Buffer at 1:100. Mix thoroughly.
3. **Wash Buffer:** Dilute 25X Wash Buffer to 1X by adding 30 ml of 25X Wash Buffer and make up the volume to 750 ml with deionized/distilled water. If crystals present in the 25X Wash Buffer, warm it in water bath at 40°C. Mix it gently. The solution must be cooled to room temperature before use.
4. **Standard Preparation:**

- Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube). Keep the tube at room temperature for 10 minutes. Mix thoroughly.
- Label 7 tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 ml of the Sample Dilution Buffer into each tube. Add 0.3 ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly.
- Transfer 0.3 ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. **Sample Dilution Buffer** was used for the blank control. (Note: Please use Standard Solutions within 2 hours of preparation).

5. Sample Preparation:

Note: Isolate the test samples soon after collecting, then, analyze immediately (within 2 hours). Or aliquot and store at -20°C (≤ 1 month) or -80°C (≤ 2 months). Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

- **Serum**: Place whole blood sample at room temperature for 2 hours or put it at 2 - 8°C overnight and centrifugation for 20 minutes at approximately 1000xg, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and endotoxin free.
- **Plasma:** Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000xg at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue homogenates**: As hemolytic blood may affect the assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01 M, pH 7.4). Mince tissue after weighing it and homogenize it in PBS (the volume depends on the weight of the tissue. Normally, 9 ml PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000xg to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
- **Cell culture supernatant**: Centrifuge supernatant for 20 minutes at 1000xg at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.
- **Cell Culture Lysate:** Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5 ml RIPA lysis buffer would be appropriate to $2x10^6$ cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3 mg.
- **Other biological fluids:** Centrifuge samples for 20 minutes at 1000xg at 4°C. Collect the supernatant and carry out the assay immediately.

Note: End user should estimate the concentration of the target protein in the test sample first, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. **The matrix components in the sample may affect the test results. Please dilute the sample ½ with Sample Dilution Buffer before testing.**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve should be run for each assay.

1. Prepare all reagents, samples (**diluted ½ with Sample Dilution Buffer**) and standards as instructed in section VII.
2. Wash plate 2 times with **1X Wash Buffer** before adding standard, sample (**diluted ½ with Sample Dilution Buffer**) and control wells.
3. Add 100 µl of each **standards** or **samples** into appropriate wells. Cover well and incubate for 1.5 hours at 37ºC.
4. Remove the cover and discard the plate content. Wash the plate 2 times with **1X Wash Buffer** without letting the wells completely dry.
5. Add 0.1 ml of **Biotin-Detection antibody** work solution into the above wells. Seal the plate and incubate at 37ºC for 60 minutes.
6. Discard the solution and wash 3 times with **1X Wash Buffer**. Wash by filling each well with Wash Buffer (350 µl) using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and then remove all residual wash-liquid from the wells by aspiration. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Tap the plate on absorbent filter papers or other absorbent materials.
7. Add 0.1 ml of **SABC working solution** into each well, cover the plate and incubate at 37ºC for 30 minutes.
8. Discard the solution and wash 5 times with **1X Wash Buffer** as step 6.
9. Add 90 µl of **TMB substrate** into each well, cover the plate and incubate at 37 ºC in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. The reaction can be terminated when apparent gradient appeared in standard wells).
10. Add 50 µl of **Stop Solution** to each well. Read result at 450 nm within 20 minutes.

IX. Calculation:

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

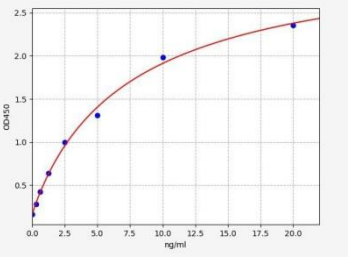

Figure: Typical Standard Curve and OD values: These standard curves are for demonstration only. A standard curve must be run with each assay.

STD. (ng/ml)	OD-1	OD-2	Average	Corrected
0	0.158	0.162	0.16	0.000
0.312	0.277	0.285	0.281	0.121
0.625	0.416	0.428	0.422	0.262
1.25	0.632	0.65	0.641	0.481
2.5	0.984	1.012	0.998	0.838
5	1.295	1.333	1.314	1.154
10	1.953	2.009	1.981	1.821
20	2.323	2.391	2.357	2.197

X. Recovery:

Matrices mentioned below were spiked with certain level of FLG and the recovery rates were calculated by comparing the measured value to the expected amount of FLG in samples

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	89-105	98
EDTA Plasma (n=5)	85-103	94
Heparin Plasma (n=5)	86-105	94

XI. Linearity:

Linearity of the assay kit was determined by spiking samples and their serial dilutions with appropriate concentration of FLG. The results are represented as percentage of calculated concentration to the expected value.

Sample	1:2	1:4	1:8
Serum (n=5)	86-104%	96-104%	89-105%
EDTA Plasma (n=5)	85-97%	86-100%	85-100%
Heparin Plasma (n=5)	80-95%	82-95%	89-99%

XII. Related Products:

- AKT1/PKB (Human) ELISA Kit (K4166)
- PADI4 (Human) ELISA Kit (E4969)
- ERK2 (Human) ELISA Kit (E4315)
- ACCPA (Mouse) ELISA Kit (E4966)
- PADI2 (Human) ELISA Kit (E4968)