



## **Shanghai Coon Koon Biotech Co., Ltd**

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### **Human Anti-Filaggrin Anti-body (AFA) ELISA Kit**

**Cat.No:** CK-bio-10343

#### **Intended use**

This assay use qualitative enzyme immunoassay method.

The microtiter plate provided has been pre-coated with antigen, add sample, HRP in turn, incubated and thoroughly washed.The Stop Solution changes the color from blue to yellow and the intensity of the color is measured at 450 nm by using spectrophotometer.

The calibration standards was assayed at the same time with the samples and allow the operator to produce a cutoff value. Then to compare with the CUTOFF value, to judge the existence or not of AFA in the samples.

## Sample collection and storages

**Serum** - Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at approximately 3000×g. Remove serum and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 30 minutes at 3000×g at 2-8°C within 30 minutes of collection. Store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.

**Cell culture supernates and other biological fluids** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20°C or -80°C. Avoid repeated freeze-thaw cycles.



**Note:** The samples should be centrifuged adequately and no hemolysis or granule was allowed.

## Materials supplied

Name	96 determinations	48 determinations
Microelisa stripplate	12*8strips	12*4strips
Negative control	0.5ml	0.5ml
Positive control	0.5ml	0.5ml
HRP-Conjugate reagent	10.0ml	5.0ml
20X Wash solution	25ml	15ml
Sample Diluent	6.0ml	3.0ml
Chromogen Solution A	6.0ml	3.0ml
Chromogen Solution B	6.0ml	3.0ml

Stop Solution	6.0ml	3.0ml
Closure plate membrane	2	2
User manual	1	1
Sealed bags	1	1

## Reagent preparation

20×wash solution:Dilute with Distilled or deionized water 1:20.

## Materials required but not supplied

1. Standard microplate reader(450nm)
2. Precision pipettes and Disposable pipette tips.
3. 37 °C incubator

## Precautions

1. Do not substitute reagents from one kit to another. Standard, conjugate and microplates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Do not remove microplate from the storage bag until needed. Unused strips should be stored at

2-8°C in their pouch with the desiccant provided.

3. Mix all reagents before using.

Remove all kit reagents from refrigerator and allow them to reach room temperature ( 20-25°C)

## Assay procedure

1. Prepare all reagents before starting assay procedure. It is recommended that all Standards and Samples be added in duplicate to the Microelisa Stripplate.
2. Separately add Positive control and Negative control 50µl to the Positive and Negative well; Add testing sample 10µl then add Sample Diluent 40µl to testing sample well.
3. Add 100µl of HRP-conjugate reagent to each well, cover with an adhesive strip and incubate for 60 minutes at 37°C.
4. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Solution (400µl) using a squirt bottle, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add chromogen solution A 50µl and chromogen solution B 50µl to each well. Gently mix and incubate for 15 minutes at 37°C. **Protect from light.**
6. Add 50µl Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

7. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader within 15 minutes.

### **Determine the result**

1. Test validity: the average of Positive control well  $\geq 1.00$ ; the average of Negative control well  $\leq 0.15$ .
2. Calculate Critical (CUT OFF): Critical = the average of Negative control well + 0.15.

Negative Result: sample OD < Calculate Critical (CUT OFF) is Negative.

Positive Result: sample OD  $\geq$  Calculate Critical (CUT OFF) is Positive.

### **Storage and validity**

Storage: 2-8°C.

validity: twelve months.

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**