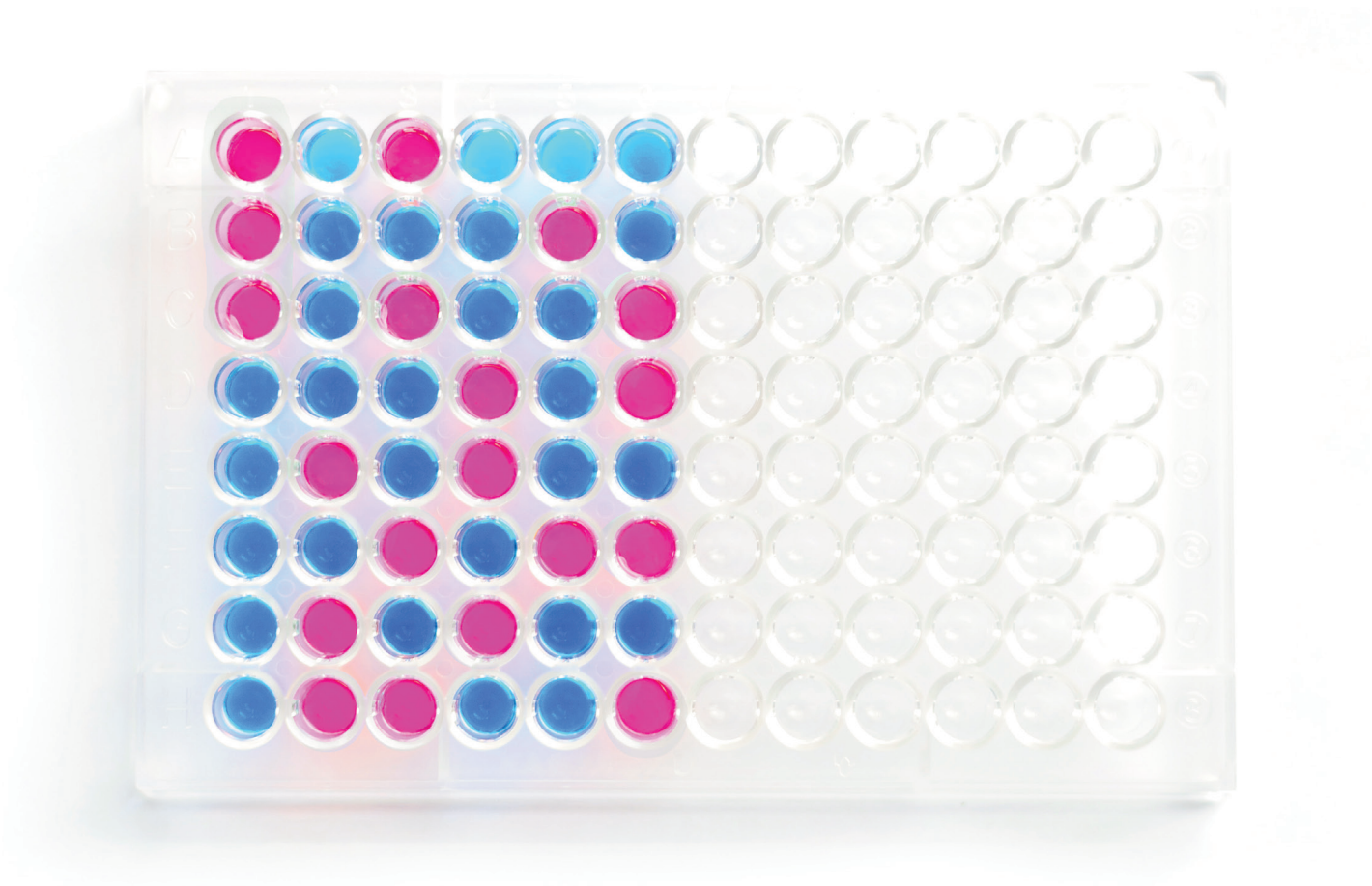
Notice
Check your package **insert & kit box for lot** specific instructions

KOMA ELISA Kit

User Manual



LABISKOMA

www.labiskoma.co.kr

Rat IgE ELISA Core Kit

Catalog No.	**K0231126**
Lot No.	**38221**
Quantity	**1000 tests**
Storage	**4°C**
Standard Range	**1.56-100 ng/ml**

[Important Notice]

- **Please read this User Manual carefully prior to performing the assay.**
- **The reagent preparation method might be different from lot to lot, so please check the lot and follow the instructions given in this manual.**
- **Do not mix or interchange reagents between different lots.**
- **The kit is intended for Research Use Only.**

Table of Contents

DESCRIPTION

This kit contains all the necessary reagents required for performing quantitative measurement of the protein from samples including serum, plasma, culture medium or other biological fluids in a sandwich ELISA format.

KIT COMPONENTS

Component	Amount
Affinity Purified Coating Antibody (Lyophilized)	10 EA
HRP-conjugated Affinity Purified Detection Antibody (Lyophilized)	10 EA
Standard Protein (Lyophilized)	10 EA
TMB or pink-ONE Solution (100 ml)	1 EA

ADDITIONAL MATERIALS REQUIRED

- ELISA microplates
- Coating Buffer
- Tween-20
- Blocking Solution
- Stop Solution
- PBS
- Plate Sealer

STORAGE AND STABILITY

- Store kit at 4°C immediately upon receipt.
- The shelf life of the kit is one year from date of shipment.
- Expiry of the kit is stated on labels.

STANDARD RANGE

Standard Range	1.56-100 ng/ml

SAMPLE PREPARATION

- **Store all samples on ice after preparation and use immediately or aliquot and store at -80°C.**
- **Avoid repeated freeze-thaw cycles.**

1) Cell culture supernatants

Centrifuge cell culture media at 1,500 rpm for 10 minutes at 4°C to remove particulates.
Immediately aliquot supernatants and store at -80°C.

2) Serum

Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3,000 rpm for 10 minutes at 4°C to remove clots. Immediately aliquot supernatants and store at -80°C.

3) Plasma

Collect plasma using anti-coagulant (citrate, EDTA or heparin). Centrifuge samples at 3,000 rpm for 15 minutes at 4°C. Immediately aliquot supernatants and store at -80°C.

REAGENT PREPARATION

- **Do not mix or substitute Assay Diluent from other kit lots.**
- **All reagents should be prepared right before use, and diluted solution should be used immediately.**

1) Buffer Preparation

Assay Diluent	PBS
Blocking Solution	PBS
Coating Buffer	50mM Carbonate-Bicarbonate Buffer, pH9.6
Washing Buffer	PBS, 0.05% Tween-20, pH7.4 Add 0.5ml Tween 20 to 1 Liter PBS and mix well.

2) Standard Protein

Reconstitute 1 vial of Standard protein in 0.11 ml sterile water to a concentration of 10,000 ng/ml. Then dilute in Assay Diluent at 1:2 serial dilutions as follows. The standard diluent buffer serves as zero standard.

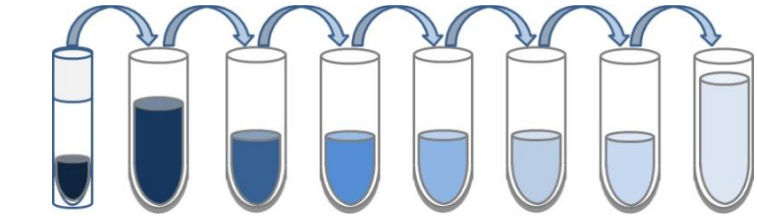


Table 1.

Step	Dilution Method	Concentration
Step A	0.01 ml of Standard + 0.99 ml of Assay Diluent	100 ng/ml
Step B	0.5 ml of Step A + 0.5 ml of Assay Diluent	50 ng/ml
Step C	0.5 ml of Step B + 0.5 ml of Assay Diluent	25 ng/ml
Step D	0.5 ml of Step C + 0.5 ml of Assay Diluent	12.5 ng/ml
Step E	0.5 ml of Step D + 0.5 ml of Assay Diluent	6.25 ng/ml
Step F	0.5 ml of Step E + 0.5 ml of Assay Diluent	3.125 ng/ml
Step G	0.5 ml of Step F + 0.5 ml of Assay Diluent	1.5625 ng/ml

3) Coating Antibody

Reconstitute 1 vial of Coating Antibody in 0.1 ml sterile water, and dilute 1:100 in Coating Buffer.

4) Detection Antibody

Reconstitute 1 vial of Detection Antibody in 0.1 ml sterile water, and dilute 1:1,000 in Assay Diluent.

NOTE: Reconstituted solutions are stable at -20°C for up to 1 month.
Do not repeat freezing and thawing.

ELISA PROCEDURE

[Caution]

When using this kit, we recommend **using a multichannel pipet instead of an ELISA washer for washing**.

Since this kit is a non-blocking type, the use of an ELISA washer can affect the background.

1) **Coating:** Add 100ul of diluted Coating Antibody to each well. Cover the plate with a plate sealer and incubate at 4°C overnight.

2) **Washing:** Add 300 ul of Washing Solution to each well. Aspirate the wells to remove liquid and wash the plate 4 times using 300 ul of Washing Solution per well. After the last wash, invert plate to remove residual solution and blot on paper towel.

NOTE: Do not let the well dry completely and go immediately to the next step.

3) **Blocking:** Add 200 ul of Blocking Solution to each well. Cover the plate with a plate sealer. Incubate at room temperature for at least 1 hour.

4) **Washing:** Aspirate the wells to remove liquid and wash the plate 4 times as in step 2.

5) **Reaction:** Add 100 ul of standard, blank and sample to each well in duplicate. Cover the plate with a plate sealer. Incubate at room temperature for at least 1 hour.

6) **Washing:** Aspirate the wells to remove liquid and wash the plate 4 times as in step 2.

7) **Detection:** Add 100 ul of the diluted detection antibody per well. Then cover the plate and incubate at room temperature for 1 hour.

8) **Washing:** Aspirate and wash plate 4 times as in step 2.

9) **Color Development:** Add 100 ul of TMB or pink-ONE TMB solution to each well. Incubate at room temperature for a proper color development. Add 100 ul of the stop solution to each well.

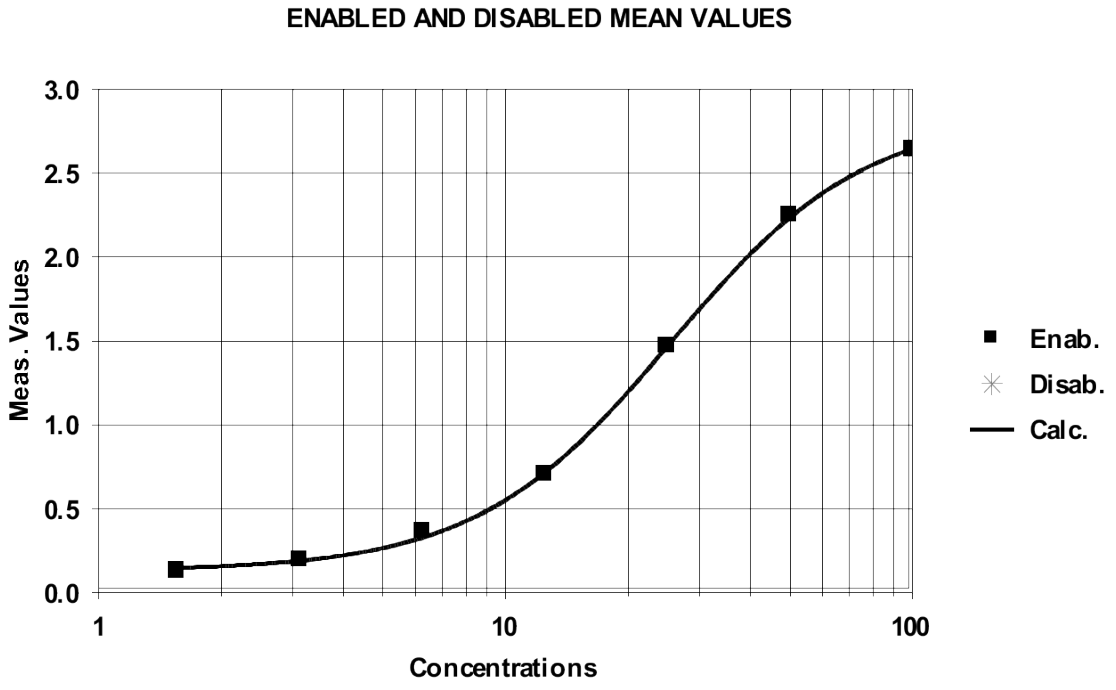
NOTE: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please monitor the color development to optimize the incubation time.

NOTE: Stop Solution (H_2SO_4) is a caustic material. Eye, hand, face and clothing protection should be worn when handling this reagent.

10) **Reading:** Using a microplate reader, measure observance at 450 nm.

CALCULATION OF RESULTS

Create a standard curve by reducing the data using ELISA reader's computer software capable of generating Standard curve-fit.
A standard curve should be generated for each set of samples.



Rat IgE (ng/ml)
(6 minutes Color development)

CROSS REACTIVITY

To define the specificity of this ELISA, several proteins were tested for cross reactivity at 50 ng/ml.

Human : N/A
Mouse : N/A
Rat : N/A

Others : N/A

TROUBLESHOOTING

Problem	Probable Cause	Solution
Low OD No Signal	Reagents not fresh or contaminated	Ensure proper preparation of reagents.
	Incubation time not long enough	Ensure sufficient incubation times.
	Incubation temperature too low	Reagent solutions should be at RT before use.
	Stop solution not added	Addition of stop solution
High OD	Inadequate standard dilution	Ensure proper dilution of Standard.
	Inadequate incubation time of detection antibody, Streptavidin-HRP or Substrate	Decrease incubation time.
Poor consistency of replicates	Inadequate washing	Increase the stringency of washes.
	Inaccurate pipetting	Ensure accurate pipetting of volume and avoid air bubbles.
	Inadequate mixing of samples	Mix samples thoroughly before pipetting
	High particulate matter of samples	Mix samples thoroughly and remove particulates by centrifugation.
	Cross-well contamination	Use fresh plate sealers or pipette tips
High background	Contamination of reagents or samples	Use a clean container before addition into wells.
	Insufficient plates washing	Ensure proper washing of each well
	Too much concentrated detection antibody and Streptavidin-HRP	Ensure proper dilution of detection antibody or conjugate and incubation time.
	Substrate solution or stop solution is not fresh	Use fresh substrate and stop solution.
	Plate left too long before reading on the plate reader	Read on the plate reader right after the experiment.
	Incubation temperature is too high	Decrease incubation temperature of substrate.
Poor standard curve	Samples contain no or below detectable levels of analyte or samples contain analyte concentrations greater than the highest standard point.	If samples are below detectable levels, higher sample volume. If samples are higher than detectable levels, it may require dilution and reanalysis.

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

CERTIFICATE OF ANALYSIS

Product	**Rat IgE ELISA Core Kit**
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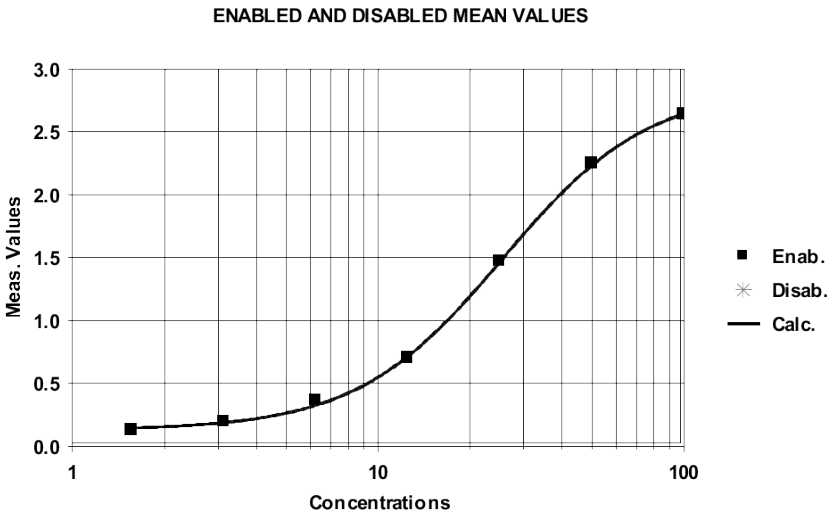

Rat IgE (ng/ml)

(6 minutes Color development)

Layout map for calibrators sheet

	Concentration		OD (450nm)
A	Cal_1	100 ng/ml	2.641
B	Cal_2	50 ng/ml	2.252
C	Cal_3	25 ng/ml	1.471
D	Cal_4	12.5 ng/ml	0.702
E	Cal_5	6.25 ng/ml	0.366
F	Cal_6	3.125 ng/ml	0.196
G	Cal_7	1.5625 ng/ml	0.13
H	Black	0 ng/ml	0.091