## **ELISA Starter Kit**

1. Catalog No. K0331001

2. Quantity

3. Storage Store at 4°C. Do not freeze.

10 plate

4. Description

- Ready to use : There is no need to prepare extra solutions separately.
- Time & Labor saving : It minimizes the effort & time to prepare the full ELISA kit.
- Colored label : It helps distinguish the solutions.
- All kit components are optimized for common ELISA test

5. Kit Contents

component	size	Cat No.
ELISA well plate	10 plate	K0331011
Coating Buffer (pH 9.6)	125 ml x 2	K0331021
Blocking Solution (0.1% Casein/PBS)	125 ml x 2	K0331031
PBS Powder	Pouch for 1L x 5	K0331041
Tween-20 (50%)	1 ml (50%) x 5	K0331051
*TMB Substrate Kit		K0331060
TMB Solution	60 ml	
Substrate $(H_2O_2)$ Solution	125 ml	
Stop Solution (2M H <sub>2</sub> SO <sub>4</sub> ) : Corrosive	60 ml	K0331081
Plate Sealing Film	10ea	

## 6. Reagent Preparation

- 1. Coating Solution: Resolve the coating material (antigen or antibody) in the coating buffer to make 1 ug/ml (1-10 ug/ml).
- Sample/Standard/Antibody Dilution: Dilute Sample/Standard/Antibody in PBS (Reconstitute 1ea PBS Powder Pouch to DW and make 1 Liter). Or use PBST (Washing solution) or Blocking solution instead of PBS to help prevent non-specific binding.
- 3. Washing Solution: Add 1vial of Tween (50% 1 ml Tween 20) to 1 Liter PBS and mix well.
- 4. Color Development Solution: Mix 1 volume of Color Development Reagent A and 2 volume of Reagent B (1:2) prior to use
- \* Note: All samples and kit reagents should be at room temperature (20-25°C) prior to use.

## 7. Procedure

- 1. Coating
  - (1) Dispense 100 ul (50-200 ul) of prepared Coating Solution to each well. (2) Incubate for overnight at  $4^{\circ}$ C.
- 2. Washing (All washing method is the same.)
  - (1) Remove the solution of each well and fill up the Washing Solution. Repeat 3-5 times. Complete removal of liquid at each step is essential to good performance.
  - (2) After the last wash, remove any remaining Washing Solution. Invert the plate and blot carefully with paper towel.

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- 3. Blocking
  - (1) Add 200 ul Blocking Solution to each well.
  - (2) Incubate 1 hour at room temperature.
- 4. Washing
- 5. React Sample/Standard (or Primary Antibody)
  - (1) Add 100 ul diluted Sample/Standard (or Primary Antibody) to each well.
  - (2) Incubate 1-2 hour at room temperature.
- 6. Washing
- 7. Add HRP-conjugated Detection Antibody (or Secondary Antibody)
- (1) Add 100 ul diluted Detection Antibody to each well.
- (2) Incubate 1-2 hour at room temperature.
- 8. Washing
- 9. Color Reaction and Reading
  - (1) Add 100 ul of color development solution to each well. Incubate at room temperature for a proper color development. (5-40 minutes)
  - (2) After sufficient color development (5-10minutes at room temperature or at  $37^{\circ}$ C), add 100 ul Stop Solution (2M H<sub>2</sub>SO<sub>4</sub>) to each well.
  - (3) Read plates in a microwell plate reader at wavelength setting of 450 nm.

## 8. Cautions

- 1. Store all solutions at  $4^{\circ}$ C and keep them from contamination.
- 2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.
- 3. Complete washing of the plate after each incubation step is essential to obtaining low background values.
- 4. Dissolve antigen, standard and antibody perfectly.
- 5. Use clean pipet tips for each transfer to avoid cross contamination.
- 6. Stop solution (2M H<sub>2</sub>SO<sub>4</sub>) is a caustic material. Eye, hand, face, and clothing protection should be worn when handling this material.
- 7. Individual components of the assay contain no preservatives except Coating Buffer and Blocking Solution. The two Solutions contain 0.02% sodium azide for longer storage. The sodium azide is also caustic material.
- \* Note: For laboratory use only. Not for diagnostic or therapeutic use.

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