# **ELISA Starter Kit, pink-ONE**

**1. Catalog No.** K0331001P

2. Quantity 10 Plate

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

**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
Pink-ONE TMB Solution	100 ml	K0331070
Stop Solution (2M H <sub>2</sub> SO <sub>4</sub> ) : Corrosive	100 ml	
Plate Sealing Film	10 ea	

## 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
- \* 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°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.
- 3. Blocking
  - (1) Add 200 ul Blocking Solution to each well.
  - (2) Incubate 1 hour at room temperature.
- Washing

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- 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) Dispense 100 ul Pink-ONE TMB Solution into each well.
  - (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 °C and keep them from contamination.
- 2. All samples and kit reagents should be at room temperature (20-25°C) prior to use.
- 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 Blocking Solution. The Blocking Solution contains 0.02% Thimerosal for longer storage. The Thimerosal is also caustic material.
- \* Note: For laboratory use only. Not for diagnostic or therapeutic use.

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