

## ELISA Starter Kit (Casein), 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.
<b>ELISA well plate</b>	10 plate	<b>K0331011</b>
<b>Coating Buffer (pH 9.6)</b>	125 ml x 2	<b>K0331021</b>
<b>Blocking Solution (0.1% Casein/PBS)</b>	125 ml x 2	<b>K0331031</b>
<b>PBS Powder</b>	Pouch for 1L x 5	<b>K0331041</b>
<b>Tween-20 (50%)</b>	1 ml (50%) x 5	<b>K0331051</b>
<b>Pink-ONE TMB Solution</b>	100 ml	<b>K0331070</b>
<b>Stop Solution (2M H<sub>2</sub>SO<sub>4</sub>) : Corrosive</b>	100 ml	<b>K0331081</b>
<b>Plate Sealing Film</b>	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).
2. 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.

\* 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.
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) Dispense 100 ul Pink-ONE TMB Solution into each well.
  - (2) After sufficient color development(5-10minutes at room temperature or at 37 °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.
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 Blocking Solution. The Blocking Solution contains 0.02% Thimerosal for longer storage. The Thimerosal is also caustic material.