RayBiotech
Empowering your proteomics

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*ISO 13485:2016*

# Custom ELISA Kit

**Catalog Number:** EL-PRELIM
User Manual
Last Revised: January 22, 2024

Protocol Video

*Please read entire manual carefully before starting experiment.*

## Introduction

The RayBio® Sandwich ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of in biological sample such as serum, plasma or cell culture supernatants. This assay employs an antibody specific capture antibody coated on a 96-well plate. Standards and samples are pipetted into the wells and specific target protein present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated specific detection antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of specific target protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

What does it mean when an ELISA has a 5-7 week lead time?
RayBiotech has a vast library of array-validated antibody pairs, however not all of them have been developed in the 96 well plate ELISA format. These ELISA kits require a 5-7 week development phase, which RayBiotech begins after receiving an order for the kit. The development only needs to occur once, after which the final kit will have passed RayBiotech's ELISA quality control tests, and will thereafter be a stock kit with normal lead times.

**Short on sample, or need higher sensitivity?** Check out the IQELISA® Immuno-PCR assay platform and our Simoa® Single Molecule Protein Detection Services.

## Storage

The entire kit may be stored at -20°C for up to 1 year from the date of shipment. Avoid repeated freeze-thaw cycles. The kit may be stored at 4°C for up to 6 months. For extended storage, it is recommended to store at -80°C. For prepared reagent storage, see table below.

## Reagents

| Component | Size / Description | Storage / Stability After Preparation |
|---|---|---|
| species target protein Microplate | 96 wells (12 strips x 8 wells) coated with anti-species target protein. | 1 month at 4°C* |
| species target protein Standard Protein | 2 vials of species target protein. 1 vial is enough to run each standard in duplicate. | 1 week at -80°C |
| species target protein Detection Antibody | 2 vials of biotinylated anti-species target protein. Each vial is enough to assay half the microplate. | 5 days at 4°C |
| Wash Buffer | 25 ml of 20X concentrated solution. | 1 month at 4°C |
| HRP-Streptavidin | 200 µl X concentrated HRP-conjugated streptavidin. | Do not store and reuse. |
| TMB One-Step Substrate Reagent | 12 ml of 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution. | N/A |
| Stop Solution | 8 ml of 0.2 M sulfuric acid. | N/A |
| Assay Diluent(s) | Diluents for samples, standard and HRP-Streptavidin | 1 month at 4°C |

*Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

## Additional Materials Required

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Log-log graph paper or computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

## Reagent Preparation

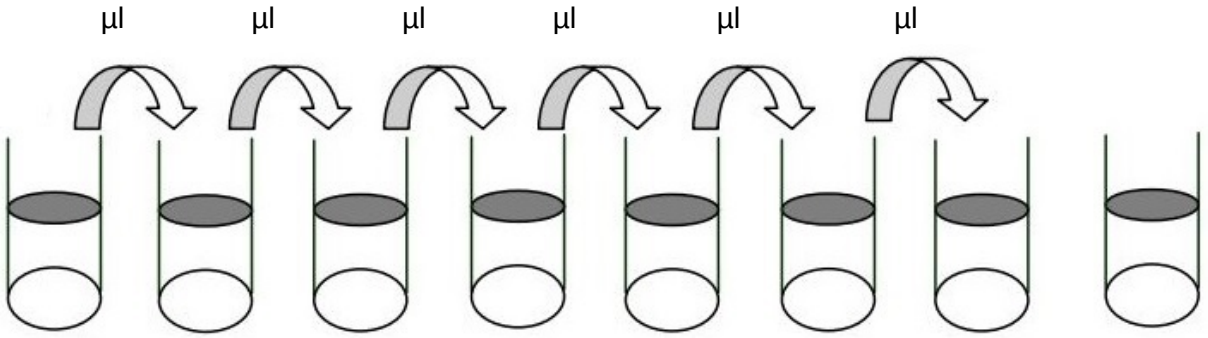
1. Bring all reagents and samples to room temperature (18 - 25°C) before use.

2. Assay Diluent B should be diluted 5-fold with deionized or distilled water before use.

3. Sample dilution: Samples should be diluted with provided Assay Diluent(s)

   **Note:** Levels of target protein may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: Approriate standard preparation will be determined during the development process.




| | Std1 | Std2 | Std3 | Std4 | Std5 | Std6 | Std7 | Zero Standard |
|---|---|---|---|---|---|---|---|---|
| **Diluent volume** | Standard + | µl | µl | µl | µl | µl | µl | µl |
| **Conc.** | | | | | | | | 0 |

5. If the Wash Buffer (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

6. Appropriate Detection Antibody preparation will be determined during the development process.

7. Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use, as precipitates may form during storage. HRP-Streptavidin concentrate should be diluted with Assay Diluent.

## Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8-well strips as appropriate for your experiment.

3. Add 100 µl of each standard (see Reagent Preparation step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature with gentle shaking.

4. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µl) using a multi-channel Pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100 µl of 1X prepared biotinylated antibody (Reagent Preparation step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µl of prepared Streptavidin solution (see Reagent Preparation step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

8. Discard the solution. Repeat the wash as in step 4.

9. Add 100 µl of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

## Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature.
3. Add 100 µl prepared biotin antibody to each well. Incubate 1 hour at room temperature.
4. Add 100 µl prepared Streptavidin solution. Incubate 45 minutes at room temperature.
5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.
6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately.

## Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

### A. Typical Data

An example of a typical standard curve for this kit will be avaliable after the development process.

### B. Sensitivity

The minimum detectable dose of the target protein will be determined during the development process.

Minimum detectable dose is define as the analyte concentration resulting in an absorbance that is 2 standard deviation higher than that of the blank (diluent buffer).

### C. Spiking & Recovery

Recovery will be determined during the development process by spiking various levels of the target protein into serum, plasma and cell culture media.

### D. Linearity

Linearity will be determined in serum, plasma and cell culture media during the development process

### E. Reproducibility

Intra-Assay CV%: <10%
Inter-Assay CV%: <12%

## Specificity

Specificity will be determined during the development process.

## Troubleshooting Guide

| Problem | Cause | Solution |
|---|---|---|
| Poor standard curve | • Inaccurate pipetting<br>• Improper standard dilution | • Check pipettes<br>• Briefly centrifuge the standard protein and dissolve the powder thoroughly by gently mixing |
| Low signal | • Improper preparation of standard and/or biotinylated antibody<br>• Too brief incubation times<br>• Inadequate reagent volumes or improper dilution | • Briefly spin down vials before opening. Dissolve the powder thoroughly.<br>• Ensure sufficient incubation time. Assay procedure step 3 may be done overnight at 4°C with gentle shaking (note: may increase overall signals including background).<br>• Check pipettes and ensure correct preparation |
| Large CV | • Inaccurate pipetting<br>• Air bubbles in wells | • Check pipettes<br>• Remove bubbles in wells |
| High background | • Plate is insufficiently washed<br>• Contaminated wash buffer | • Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.<br>• Make fresh wash buffer |
| Low sensitivity | • Improper storage of the ELISA kit<br>• Stop solution | • Store your standard at <-70°C after reconstitution, others at 4°C. Keep substrate solution protected from light.<br>• Add stop solution to each well before reading plate |