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ISO 13485:2016 cGMP

RayBio® Anti-Drug Antibody ELISA Kit

Catalog #: EAD-ADA-PRELIM

User Manual
Last Revised: March 13, 2025

Introduction

The RayBio® Anti-Drug Antibody ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of anti-drug antibodies (ADA) in serum, plasma, and cell culture supernatants. This assay employs the drug antibody coated on a 96-well plate. Standards and samples are pipetted into the wells and ADA present in the sample are bound to the wells by antibody drug. The wells are washed and Biotinylated Drug Antibody is added. After washing away unbound Biotinylated Drug 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 ADA bound. The stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Storage / Stability

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.

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Kit Components

Name	Catalog #	Size / Qty	Description	Storage / Stability After Preparation
DA Microplate	EAD-ADA-A	96 wells	96 wells (12 strips x 8 wells) coated with Drug Antibody	1 month at -20°C*
Anti-DA Standard	EAD-ADA-STD	2 vials	Anti-drug antibody standard. 1 vial is enough to run each standard in duplicate.	1 week at -20°C
DA Detection Antibody	EAD-ADA-DAB	2 vials	Biotinylated Drug Antibody. 1 vial is enough to assay half the microplate.	5 days at 4°C
HRP-Streptavidin	EAD-HRP	600 µl	Concentrated HRP-conjugated streptavidin.	Do not store and reuse.
Wash Buffer	EL-ITEMB	25 ml	20X concentrated wash buffer	1 month at 4°C
Assay Diluent	EL-ITEME	15 ml	5X concentrated assay diluent	1 month at 4°C
TMB One-Step Substrate Reagent	EL-TMB	12 ml	3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution	N/A
Stop Solution	EL-STOP	8 ml	0.2 M sulfuric acid	N/A

*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. Horizontal orbital microplate shaker.
9. Tubes to prepare standard or sample dilutions.

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Reagent Preparation

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use.
2. Sample dilution: 1X Assay Diluent should be used for dilution of serum, plasma, and cell culture supernatant samples.

   Note: Levels of Anti-Drug Antibody may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.
3. Appropriate standard preparation will be determined during the development process.
4. If the Wash Concentrate (20X) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.
5. Appropriate Detection Antibody preparation will be determined during the development process.
6. Appropriate HRP- Streptavidin preparation will be determined during the development process.

Assay Procedure

1. Bring all reagents and samples to room temperature (18 - 25ºC) before use. It is recommended to run all standards and samples in at least duplicate.
2. Label removable 8-well strips as appropriate for your experiment.
3. Add 100 µl of each standard and sample into appropriate wells. Cover wells and incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500 rpm.
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 auto-washer. Complete removal of liquid at each step is essential for 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 prepared 1X biotinylated anti-DA detection antibody to each well. Incubate for 1 hour at room temperature on the shaker.
6. Discard the solution. Repeat the wash as in step 4.

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7. Add 100 µl of prepared HRP-Streptavidin solution to each well. Incubate for 45 minutes at room temperature on the shaker.
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 on the shaker.
10. Add 50 µl of Stop Solution to each well. Read at 450 nm immediately.

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Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.

2. Add 100 µl standard or sample to each well. Incubate 2 hours at room temperature on the shaker.

3. Add 100 µl prepared detection antibody to each well. Incubate 1 hour at room temperature on the shaker.

4. Add 100 µl prepared HRP-Streptavidin solution. Incubate 45 minutes at room temperature on the shaker.

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature on the shaker.

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

Typical Data

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. Standard Curve

These standard curves are for demonstration only. A standard curve must be run with each assay.

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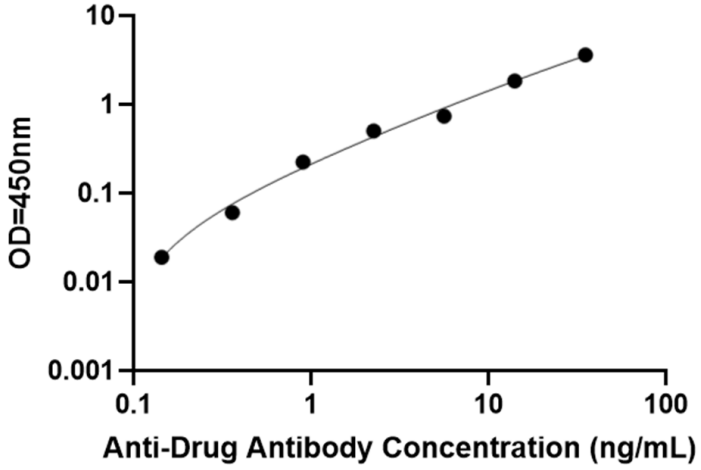

B. Sensitivity

The minimum detectable dose of Anti-Drug Antibody will be determined during the development process.

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

C. Spiking & Recovery

Recovery will be determined by spiking various levels of Anti-Drug Antibody into human serum, plasma and cell culture media samples.

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

D. Reproducibility

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

E. Specificity

This ELISA antibody pair detects Anti-Drug Antibody. Other targets not determined.

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Troubleshooting Guide

Problem	Cause	Solution
Low signal in samples	• Sample concentration is too low • Improper preparation of detection antibody • Too brief incubation times • Inadequate reagent volumes or improper dilution	• Increase sample concentration Briefly spin down vials before opening. Dissolve the powder thoroughly. • Ensure sufficient incubation time; assay procedure step 3 may be done overnight Check pipettes and ensure correct preparation
High signal in samples	• Sample concentration is too high	• Reduce sample concentration
Large CV	• Inaccurate pipetting Air bubbles in wells	• Check pipettes • Remove bubbles in wells
High background	• Plate is insufficiently washed • Contaminated wash buffer	• Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed. • Make fresh wash buffer
Low sensitivity	• Improper storage of the ELISA kit • Stop solution • Improper primary or secondary antibody dilution	• Store your standard at <- 70ºC after reconstitution, others at 4ºC. Keep substrate solution protected from light. • Add stop solution to each well before reading plate • Ensure correct dilution