

Product Information

ELISA Kit Information

Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

This ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of a target protein in biological samples, such as serum, plasma, cell culture supernatants, urine, and/or cell and tissue lysates (see current Certificate of Analysis for validated sample types). This assay employs a specific capture antibody coated on a 96 well plate. Standards and samples are pipetted into the wells and the target protein present in a sample is bound to the wells by the immobilized antibody. The wells are washed and a biotinylated detection antibody specific for the target protein 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 target protein bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Antibody-coated ELISA Plate (Item A) - 96 wells (12 strips x 8 wells) coated with specific capture antibody.
2. 20x Wash Buffer (Item B) – RABWASH4: 25 ml of 20x concentrated solution.
3. Target Protein Standard (Item C) - 2 vials, recombinant protein.
4. Assay/Sample Diluent Buffer/s: See current [Certificate of Analysis](#)
5. Biotinylated Detection Antibody (Item F) 2 vials of biotinylated detection antibody (each vial is enough to assay half a microplate).
6. HRP-Streptavidin (Item G) – RABHRP5: 200 µl of concentrated HRP-conjugated streptavidin.
7. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) – RABTMB3: 12 ml of 3, 3', 5, 5'-tetra-methylbenzidine (TMB) in buffer solution.
8. ELISA Stop Solution (Item I) – RABSTOP3: 8 ml of 0.2 M sulfuric acid.

Reagents and Equipment Required but Not Provided.

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. SigmaPlot® software (or other software which can perform four-parameter logistic regression models).
8. Tubes to prepare standard or sample dilutions.

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

1. Bring all reagents and samples to room temperature (18–25 °C) before use.
2. Assay/Sample Diluent Buffer dilution: See current [Certificate of Analysis](#) for dilution instructions.
3. Sample dilution: See current [Certificate of Analysis](#) for dilution instructions and recommendations. **Note:** Levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.
4. Preparation of target protein standards: See current [Certificate of Analysis](#) for dilution instructions.
5. If the Wash Buffer (20x) (Item B) 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.

6. Preparation of Biotinylated Detection Antibody: See current Certificate of Analysis for dilution instructions.
7. Dilution of HRP-Streptavidin concentrate (Item G): See current Certificate of Analysis for dilution instructions.

Storage/Stability

Store the kit at $-20\text{ }^{\circ}\text{C}$. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at $-20\text{ }^{\circ}\text{C}$ or $-70\text{ }^{\circ}\text{C}$ ($-70\text{ }^{\circ}\text{C}$ is recommended). Opened microplate strips or reagents may be store for up to 1 month at $2-8\text{ }^{\circ}\text{C}$. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure

1. Bring all reagents and samples to room temperature ($18-25\text{ }^{\circ}\text{C}$) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add $100\text{ }\mu\text{l}$ of each standard (see Preparation, step 4) and sample into appropriate wells. Cover wells and incubate for 2.5 hours at room temperature or overnight at $4\text{ }^{\circ}\text{C}$ with gentle shaking.
3. Discard the solution and wash 4 times with 1x Wash Solution. Wash by filling each well with Wash Buffer ($300\text{ }\mu\text{l}$) using a multichannel 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.
4. Add $100\text{ }\mu\text{l}$ of 1x prepared Biotinylated Detection Antibody (see Preparation, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add $100\text{ }\mu\text{l}$ of prepared HRP-Streptavidin solution (see Preparation, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.
7. Discard the solution. Repeat the wash as in step 3.
8. Add $100\text{ }\mu\text{l}$ of ELISA Colorimetric TMB Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add $50\text{ }\mu\text{l}$ of Stop Solution (Item I) to each well. Read at 450 nm immediately.

ResultsCalculations

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 using SigmaPlot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit curve through the standard points.

Appendix
Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Inaccurate pipetting	Check pipettes
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at ≤ -20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Stop solution should be added to each well before measurement.

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