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Enzyme-linked Immunosorbent Assay (ELISA)

Materials

1) 96-well plate
2) Target antibody sample
3) Calibrated standard of target antibody
4) Purified antigen that target antibody will bind to
5) Labeled secondary antibody that will bind to target antibody
6) Coating buffer (50 mM Sodium Carbonate, pH 9.5)
7) Wash buffer (10mM Tris, 1M Sodium Chloride, pH 7.2, 0.05% (v/v) Kathon)
8) Blocking buffer (10 mM Tris, pH 7.2, 10% (w/v) D-Gluconic Acid, 5% (w/v) Bovine serum albumin)
9) Antibody diluent (PBS)
10) Colorimetric substrate
11) Stop solution
12) ELISA plate reader

Antigen Coating

1) Prepare a 1 µg/ml dilution of the purified antigen, using the coating buffer as the diluent.
2) Add 100 µl of the diluted antigen to as many wells of the plate as needed. If using a standard curve, 33 wells will be needed for 11 dilutions tested in triplicate. Wells will also be needed to test at least 2 dilutions of the unknown sample.
3) Add 100 µl of coating buffer (without antigen) to a set of wells for the standard curve and for the unknown sample as a negative control.
4) Incubate the plate for 3 hours at room temperature, or overnight at 4°C.
5) Flood each well of the plate with wash buffer and flick out the liquid. Wash the plate 3 times and pat dry on a paper towel.

Blocking

1) Add 375 µl of blocking buffer to each well.
2) Incubate the plates for 2 hours at room temperature, or 4-24 hours at 4°C.
3) Flick out the blocking buffer and pat the plate dry. Blocked plates can be stored covered at 4°C for up to two weeks.

Unknown Antibody and Standard Curve

1) To prepare a standard curve, dilute the control antibody to 1 µg/ml in PBS. Prepare serial dilutions ranging from 1 µg/ml to 0.0039 µg/ml. Include a blank of PBS as a negative control. Add 100 µl of each dilution into the coated wells. Each dilution (including the blank) should be added in triplicate.
2) Dilute the antibody sample to appropriate dilutions in PBS. Add 100 µl of each dilution of the test sample to coated wells. Include a blank as a negative control.
3) Incubate the plate for one hour at room temperature.
4) Wash the plate 3 times with wash buffer and pat dry.

Labeled Secondary Antibody

1) Dilute the labeled secondary antibody to 0.5 µg/ml in PBS. Add 100 µl of the diluted antibody to each well.
2) Incubate the plate for 60 minutes at room temperature.
3) Wash the plate 5 times with wash buffer and pat dry.

Antibody Detection and Plate Reading

1) Immediately before use, prepare the colorimetric reagent and add 100 µl to each well. Incubate until color develops, and stop the reaction with the appropriate solution. For example, if an HRP conjugate is used as the labeled secondary antibody, prepare a 1:1 mixture of TMB Solution and Peroxide Solution. Add 100 µl of the mixture to each well and incubate for approximately 15 minutes until the wells turn blue. Stop the reaction by adding 100 µl of 2N Sulfuric Acid to each well.
2) Read the plate at an appropriate wavelength. For HRP, read the plate at 450 nm.
3) Plot the absorbance of the standard antibody dilutions against their concentrations and draw a line of best fit.
4) Compare the absorbance of the test sample to the standard curve to determine the concentration. Multiply the values by the appropriate dilution factor.

Troubleshooting

1) Negative control gives positive results- Check for contamination of the substrate solution, the labeled secondary antibody, or the controls.
2) No color in the positive controls or samples- Check the expiration dates, concentrations, and storage conditions of the reagents.

3) Very little color in the positive controls or samples- Check the dilution of the labeled secondary antibody and the concentration of the substrate.
4) Color in the test samples but not in the positive controls – Check the expiration dates and storage conditions of the positive controls.
5) Color is seen but absorbance is lower than expected – Check the wavelength setting.