Version 26-04/25

User's Manual

KLH IgG (Human) ELISA Kit

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit allows quantitative measurement of human anti-KLH IgG levels in serum, plasma, and other fluids.

Principles of Testing

The assay uses KLH as capture reagent coated on microtiter wells, and horseradish peroxidase (HRP) conjugated anti-human IgG for detection. Standards and diluted samples are incubated in microtiter wells for 45 minutes. The wells are subsequently washed. HRP conjugate is added and incubated for 45 minutes. Anti-KLH IgG molecules are thus sandwiched between immobilized KLH and the HRP conjugate. The wells are then washed to remove unbound HRP-labeled antibodies. TMB is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of stop solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of anti-KLH IgG is proportional to the absorbance and is derived from a standard curve.

Reagents And Materials Provided

1. KLH Coated 96-well plate (12 x 8 well strips)
2. Anti-Human IgG HRP Conjugate, 11 ml
3. Anti-KLH IgG Stock (lyophilized)
4. 20x Wash Solution, 50 ml
5. Diluent, 50 ml
6. TMB Reagent, 11 ml
7. Stop Solution (1N HCl), 11 ml

Materials Required But Not Supplied

Precision pipettes and tips

Distilled or deionized water

Polypropylene or glass tubes

Vortex mixer

Absorbent paper or paper towels

Plate incubator/shaker with mixing speed of 150 rpm

Plate washer

Plate reader with an absorbance range of 0-4 at 450 nm

Graphing software

Storage

The anti-KLH IgG stock should be stored at - 20°C or lower. The remainder of the kit should be stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. The kit will remain stable for six months from the date of purchase provided that the components are stored as described.

Specimen Collection And Preparation

The optimal sample dilution should be determined empirically. Studies at Creative Diagnostics, using ascites fluid samples, suggest that a 500-fold dilution is a reasonable starting point.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (25°C) before use.
2. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
3. Wash Solution Preparation: The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.
4. Standard Preparation:

1) Standards should be used within 30 min of preparation.
2) The anti-KLH IgG stock is provided in lyophilized form. Reconstitute as directed on the vial label (the reconstituted stock should be frozen at - 20°C if additional use is intended).
3) Label 6 polypropylene or glass tubes as 30, 15, 7.5, 3.75, 1.88 and 0.94 ng/ml.
4) Into the tube labeled 30 ng/ml, pipette the volume of diluent detailed on the stock vial label. Then add the indicated volume of anti-KLH IgG and mix gently. This provides the 30 ng/ml standard.
5) Dispense 250 µl of diluent into the tubes labeled 15, 7.5, 3.75, 1.88 and 0.94 ng/ml.
6) Prepare a 15 ng/ml standard by diluting and mixing 250 µl of the 30 ng/ml standard with 250 µl of diluent in the tube labeled 15 ng/ml.
7) Similarly prepare the remaining standards by serial dilution.

Assay Procedure

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells.
3. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.
4. Aspirate the contents of the microtiter wells and wash the wells five times with 1x wash solution using a plate washer (400 µl/well).
5. Strike the wells sharply onto absorbent paper to remove all residual wash solution.
6. Add 100 µl of diluted HRP conjugate into each well.
7. Incubate on a plate shaker at 150 rpm/25°C for 45-minutes.
8. Wash as detailed above.
9. Dispense 100 µl of TMB into each well.

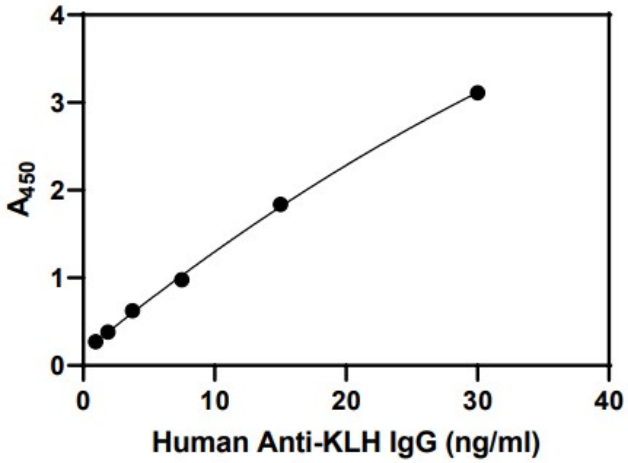
10. Incubate on a plate shaker at 150 rpm/25°C for 20-minutes.
11. Stop the reaction by adding 100 µl of stop solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Measure absorbance at 450 nm with a microtiter plate reader within five minutes.

Calculation

1. Using graphing software construct a standard curve by plotting the absorbance of the standards versus concentration.
2. Fit standard data to a two-site, total and non-specific binding model (others may be used at the discretion of the researcher) and derive the concentration of anti-KLH IgG in the samples.
3. Multiply the derived concentration by the dilution factor(s) to determine the actual concentration of anti-KLH IgG in the original sample.
4. If absorbance values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

Typical Standard Curve

A typical standard curve is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns.



Anti-KLH IgG (ng/ml)	A_{450}
30	3.110
15	1.837
7.5	0.977
3.75	0.624
1.88	0.381
0.94	0.271

Performance Characteristics

Parallelism: To assess performance of the assay, two samples containing anti-KLH IgG at concentrations of 10.9 and 1.4 µg/ml were serially diluted from 100- to 6400-fold to produce values within the dynamic range of the assay.