Version 18-04/25

User's Manual

KLH IgG (Guinea Pig) ELISA Kit

REF **DEIA3833**

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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.

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PRODUCT INFORMATION

Intended Use

Measurement of KLH (keyhole limpet hemocyanin) induced anti-KLH antibody levels allows quantitative evaluation of the immune response. This ELISA is designed for the rapid and quantitative measurement of guinea pig anti-KLH IgG levels in serum or plasma.

Principles of Testing

The KLH IgG (Guinea Pig) ELISA Kit is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses KLH for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-guinea pig IgG (Fc specific) for detection. Serum or plasma samples are diluted and incubated in the microtiter wells for 1 hour. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. Anti-KLH IgG molecules are thus sandwiched between immobilized KLH and the detection antibody conjugate. The wells are then washed to remove unbound HRP-labeled antibodies, and TMB Reagent 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, and optical density is measured spectrophotometrically at 450 nm. The concentration of anti-KLH IgG is proportional to the optical density of the test sample.

Reagents And Materials Provided

1. KLH-coated 96-well plate: 96 (8×12) wells
2. Enzyme Conjugate Reagent: 11 mL
3. Reference standard (lyophilized)*: 1 vial
4. 20× Wash Solution: 50 mL
5. Diluent: 60 mL
6. TMB Reagent (One-Step): 11 mL
7. Stop Solution (1 N HCl): 11 mL

*Guinea pig anti-KLH IgG levels are measured in nominal units and are calibrated with reference anti-KLH guinea pig serum.

Materials Required But Not Supplied

1. Precision pipettes and tips
2. Distilled or deionized water
3. Polypropylene or glass tubes
4. Vortex mixer
5. Absorbent paper or paper towels
6. Micro-Plate incubator/shaker mixing speed of ~150 rpm
7. Plate washer

8. Plate reader with an optical density range of 0-4 at 450 nm ·
9. Graph paper (PC graphing software is optional)

Storage

1. The reference standard should be stored at -20°C for optimal stability.
2. All remaining kit components should be stored at 2-8°C
3. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. Test kit will remain stable for six months from the date of purchase provided that the components are stored as described above.

Specimen Collection And Preparation

Anti-KLH IgG is present in serum from KLH immunized guinea pigs at concentrations of ~3 million u/mL. In order to obtain values within range of the standard curve, we suggest that samples be diluted 50,000 fold using the following procedure for each sample to be tested:

1. Dispense 249 µL and 398 µL of diluent into separate tubes.
2. Pipette and mix 1.0 µL of the serum sample into the tube containing 249 µL of diluent. This provides a 250 fold diluted sample.
3. Mix 2.0 µL of the 250 fold diluted sample with the 398 µL of diluent in the second tube. This provides a 50,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

Reagent Preparation

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.

Standard Preparation

1. The guinea pig anti-KLH IgG standard is provided as a lyophilized stock. Reconstitute with 100 µL of distilled or deionized water (the reconstituted standard is stable at 4°C for one week but should be aliquoted and frozen at -20°C after reconstitution if future use is intended).
2. Label 5 polypropylene or glass tubes as 100, 50, 25, 12.5 and 6.25 units/mL (u/mL)
3. Into the tube labeled 100 u/mL, pipette the volume of diluent detailed on the anti-KLH IgG standard vial label. Then add the indicated volume of anti-KLH IgG standard (shown on the anti-KLH IgG standard vial label) and mix gently. This provides the 100 u/mL standard.
4. Dispense 250 µL of diluent into the tubes labeled 50, 25, 12.5, and 6.25 u/mL.
5. Prepare a 50 u/mL standard by diluting and mixing 250 µL of the 100 u/mL standard with 250 µL of diluent in the tube labeled 50 u/mL.
6. Similarly prepare the 25, 12.5, and 6.25 u/mL 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 (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 1 hour.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µL/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µL of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 30 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µL of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (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. Read the optical density at 450 nm with a microtiter plate reader within 5 minutes.

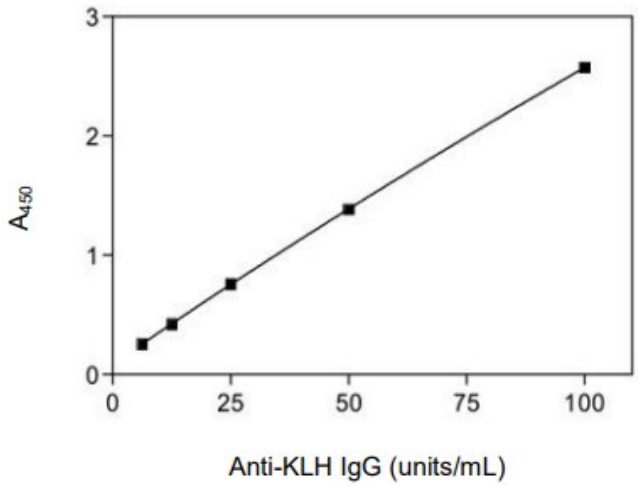
Calculation

1. Calculate the average absorbance values (A450) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/mL on linear graph paper, with absorbance on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-KLH IgG in u/mL from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of anti-KLH IgG in the serum/plasma sample.
5. PC graphing software may be used for the above steps.
6. If the OD450 values of samples fall outside the standard curve when tested at a 20,000 fold dilution, samples should be diluted appropriately and re-tested.

Typical Standard Curve

A typical standard curve with optical density readings at 450 nm on the Y axis against anti-KLH IgG concentrations on the X axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

Anti-KLH IgG (u/mL)	Absorbance (450 nm)
100	2.574
50	1.384
25	0.759
12.5	0.422
6.25	0.254



Precautions

1. Please read and understand the instructions thoroughly before using the kit.
2. This kit is intended for measurement of anti-KLH IgG levels in guinea pig serum or plasma obtained 21-days after immunization with KLH.
3. All reagents should be allowed to reach room temperature (25°C) before use.
4. The optimal sample dilution should be determined empirically. However, studies indicate that an initial sample dilution of 50,000 fold works well for most 21-day post-immunization samples. Please do not use dilutions less than 100-fold.
5. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
6. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
7. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.