Stellar KLH™ ELISA Test Kit

Rat Anti-KLH IgG ELISA Kit

Stellar Biotechnologies, Inc., Product Code ELI-02G

ELISA for the Quantitative Determination of **Rat Anti-KLH IgG** in Serum or Plasma

INTRODUCTION

Keyhole Limpet Hemocyanin (KLH) is a large oxygen-carrying, copper-containing glycoprotein from the marine mollusk *Megathura crenulata*. KLH is well known as a potent stimulator of humoral and cellular immune responses. It is widely used in research and clinical studies including, for example, as a carrier of low molecular weight haptens used in vaccines and as an antigen for assessing immune function in the screening of drug candidates.

Stellar KLH is manufactured by Stellar Biotechnologies, Inc. directly from controlled, land-based aquaculture. Stellar has developed industry-leading sustainable practices that protect the source species *Megathura crenulata* and ensure quality, consistent KLH.

In drug screening applications, determination of a drug candidate's effects on anti-KLH antibody levels allows easy assessment of immune system regulation. Animals are immunized with KLH while undergoing drug treatment and serum is collected at appropriate times post-immunization. Typically, serum collected 5-7 days after immunization is used for measurement of anti-KLH IgM levels, and serum collected 14+ days post immunization is used to measure anti-KLH IgG levels. Comparison of anti-KLH IgM or IgG levels in drug-treated vs. control groups reveals effects on immune response.

This Rat Anti-KLH IgG ELISA Kit is made using Stellar KLH and is suitable for rapid and quantitative measurement of anti-KLH IgG levels in serum or plasma.

PRINCIPLE OF THE TEST

This Rat anti-KLH IgG ELISA is a solid phase enzyme-linked immunosorbent assay. It uses Stellar KLH for solid phase (microtiter wells) immobilization, and a horseradish peroxidase (HRP) conjugated goat anti-rat IgG antibody for detection. The use of Stellar KLH improves performance of the assay. Importantly, Stellar KLH coated plates can be used to detect anti-KLH IgG in animals immunized with either subunit or whole molecule KLH.

Serum or plasma samples are diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 45 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 450nm. The concentration of anti-KLH IgG is proportional to the optical density.

KIT COMPONENTS

Materials provided with the kit:

- Stellar KLH coated 96-well plate (12 strips of 8 wells)
- Anti Rat IgG HRP Conjugate, 11 ml
- Anti-KLH IgG Stock[A] (lyophilized)
- 20x Wash Solution, 50 ml
- Diluent (50 ml)
- TMB Reagent (One-Step) 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

STORAGE OF THE TEST KIT

On receipt, the anti-KLH IgG standard stock should be stored frozen at -20°C or lower. The remainder of the kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air. **DO NOT FREEZE THE HRP CONJUGATE OR TMB SOLUTIONS**. Test kits will remain stable for six months from the date of purchase provided that the components are stored as described.

GENERAL INSTRUCTIONS

1. Please read the instructions thoroughly before using the kit.
2. All reagents should be removed from storage conditions and allowed to reach room temperature (18-25°C) before use.
3. The optimal sample dilution should be determined empirically. Do not use dilutions less than 100-fold (i.e., do not use dilutions of 50-fold).
4. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

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

5. Working 500 – 15.63 ng/ml anti-KLH IgG standards should be used within 1 hour of preparation.
6. The anti-KLH IgG stock is provided in lyophilized form. Reconstitute as directed on the vial label *(the reconstituted standard should be aliquoted and frozen at -20°C after reconstitution if additional use is intended)*.

[A] The reference standard provided with the kit was calibrated using affinity purified rat anti-KLH IgG.

7. Label 6 polypropylene or glass tubes as 500, 250, 125, 62.5, 31.25, and 15.63 ng/ml.
8. 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 stock (also detailed on the vial label) and mix gently. This provides the 30 ng/ml standard.
9. Dispense 250 µl of diluent into the tubes labeled 250, 125, 62.5, 31.25, and 15.63 ng/ml.
10. Prepare a 250 ng/ml standard by diluting and mixing 250 µl of the 500 ng/ml standard with 250 µl of diluent in the tube labeled 250 ng/ml.
11. Similarly prepare the 125, 62.5, 31.25, 15.63 ng/ml standards by serial dilution.

SAMPLE PREPARATION

The optimal sample dilution should be determined empirically. However, studies at Stellar Biotechnologies, Inc., suggest that a 2000-fold dilution is a reasonable starting point. In order to achieve high dilutions we suggest that a serial dilution strategy be used. If, for example, a 2000-fold sample dilution is desired the following procedure should used. This approach minimizes diluent usage and favors accurate and precise sample dilution.

12. Dispense 1000 µl and 1998 µl of diluent into separate tubes.
13. Pipette and mix 2 µl of the serum/plasma sample into the tube containing 1998 µl of diluent. This provides a 1000 fold diluted sample.
14. Mix 1000 µl of the 1000 fold diluted sample with the 1000 µl of diluent in the second tube. This provides a 2000 fold dilution of the sample.
15. Repeat this procedure for each sample to be tested.

Do not use dilutions less than 100-fold (i.e., do not use dilutions of 50-fold).

ASSAY PROCEDURE

16. Secure the desired number of coated wells in the holder.
17. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
18. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
19. 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.
20. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
21. Add 100 µl of HRP conjugate into each well.
22. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
23. Wash as detailed in 4 to 5 above.
24. Dispense 100 µl of TMB Reagent into each well.
25. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
26. Stop the reaction by adding 100 µl of Stop Solution to each well.
27. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
28. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

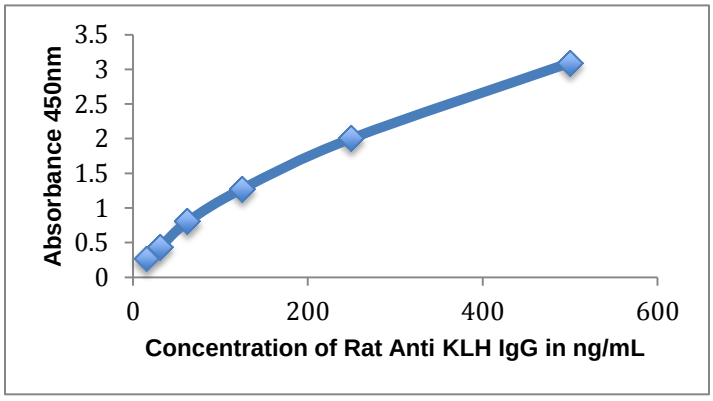
CALCULATION OF RESULTS

29. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
30. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
31. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-KLH IgG in ng/ml from the standard curve.
32. Multiply the derived concentrations by the dilution factor to determine the actual concentration of anti-KLH IgG in the serum/plasma sample.
33. PC graphing software may be used for the above steps.
34. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm 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 (ng/ml)	Absorbance (450 nm)
500	3.096
250	2.003
125	1.276
62.5	0.802
31.25	0.438
15.63	0.269



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