Monkey Anti-KLH IgG ELISA

INTRODUCTION

Drug candidates are routinely screened for evidence of immune system regulation during the discovery process. It is important that the immune response is not enhanced or diminished since such effects may lead to hypersensitivity or increased susceptibility to infection. Determination of a drug candidate's effects on anti-KLH antibody levels allows easy assessment of immune system regulation.[1] 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 IgG or IgM levels in drug treated, versus control groups reveals effects on the immune response.

PRINCIPLE OF THE ASSAY

The monkey anti-KLH IgG ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA). It uses KLH for solid phase (microtiter wells) immobilization and a horseradish peroxidase (HRP) conjugated goat anti-monkey IgG Fc antibody for detection. 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 450 nm. The concentration of anti-KLH IgG is proportional to the optical density.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- KLH coated 96-well plate (provided as 12 strips of 8 wells)
- Anti Monkey IgG HRP Conjugate, 11 ml
- Anti-KLH IgG Stock (lyophilized)[A]
- 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 450 nm
- Graph paper (PC graphing software is optional)

STORAGE

The test 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. Test kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. Please read the instructions thoroughly before using the kit.
2. All reagents should be 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

1. 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)***.
2. Label 6 polypropylene or glass tubes as 30, 15, 7.5, 3.75, 1.88, and 0.938 ng/ml.
3. 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.
4. Dispense 250 µl of diluent into the tubes labeled 15, 7.5, 3.75, 1.88, and 0.938 ng/ml.
5. 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.
6. Similarly prepare the 7.5, 3.75, 1.88, and 0.938 ng/ml standards by serial dilution.

SAMPLE PREPARATION

The optimal sample dilution should be determined empirically. However, studies at Life Diagnostics, Inc. suggest that a 10,000 fold dilution is a reasonable starting point. A 10,000 fold sample dilution may be achieved using the following procedure:

1. Dispense 495 µl and 297 µl of diluent into separate tubes.
2. Pipette and mix 5 µl of the serum/plasma sample into the tube containing 495 µl of diluent. This provides a 100 fold diluted sample.

[A] The reference standard provided with the kit is of human origin and was calibrated using pure rhesus IgG. The use of human IgG as standard allows export of the kit without requirement for CITES documentation.

3. Mix 3 µl of the 100 fold diluted sample with 297 µl of diluent in the second tube. This provides a 10,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

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 (18-25°C) for 45 minutes.
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 HRP conjugate into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 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 (18-25°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*.

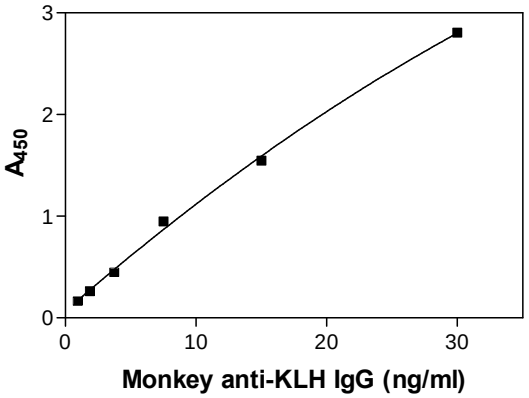
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. 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.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of anti-KLH IgG in ng/ml from the standard curve.
4. Multiply the derived concentrations 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 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)	A_{450}
30	2.807
15	1.546
7.5	0.948
3.75	0.449
1.88	0.265
0.94	0.166



REFERENCES

1. JR Picotti et.al. T-cell-dependent antibody response: Assay development in cynomolgus monkeys. Journal of Immunotoxicology, 2:191-196 (2005)

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For technical assistance please email us at
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