Guinea Pig Interleukin 6 ELISA

Catalogue #GS0270

1. Purpose

The purpose of this method is to describe testing of Guinea Pig (*Cavia porcellus*) samples for the presence of Interleukin 6 using a polyclonal sandwich ELISA.

2. Scope

This document describes the testing of samples using a purified polyclonal antibody and a biotinylated form of monoclonal antibody in a sandwich ELISA. This method is intended for research purposes only.

3. Responsibility

It is the responsibility of the personnel using this method to have read and understood the protocol and to be fully trained on safe use of laboratory equipment and reagents.

4. Materials Provided

Coating Antibody-Polyclonal rabbit anti Guinea Pig IL6 antibody
Detection Antibody-Biotinylated monoclonal rabbit anti Guinea Pig IL6 antibody
Standard-Purified Guinea Pig IL6 protein

5. Other materials and solutions required

Phosphate Buffered Saline (PBS)
Bovine Serum Albumin (BSA)
Tween 20
Streptavidin-HRP
3,3´,5,5´-tetramethylbenzidine (TMB)
2M Sulfuric Acid
microplate reader
96 well plates
Plate sealers
0.22uM filters

6. Reagent Preparation

Kit Components: Thaw kit components on ice and aliquot. Avoid freeze/thaw. Do not store the purified protein at 4ºC for more than one week.

Buffers to prepare in advance:

Block Buffer: 1% BSA in PBS. Dissolve BSA in 1xPBS at a ratio of 1g BSA per 100mL PBS. Filter sterilize and aliquot. Store at 4ºC or freeze aliquots and thaw day of use.

Sample Dilution Buffer: 1xPBS, 1%BSA, 0.5%T20. Dissolve BSA in 1x PBS at a ratio of 1g BSA per 100mL PBS. Add 0.5mL of Tween 20 per 100mL PBS. Filter sterilize and aliquot. Store at 4ºC.

Wash Buffer: 1xPBS: 1xPBS+ 0.5% Tween-20. Add 0.5mL of Tween 20 per 100mL PBS. Filter sterilize. Wash Buffer can be stored at room temperature.

7. Lot Specific Dilution Instructions

Coating Antibody-GS0227 Lot#A339: Dilute the coating antibody just prior to use in the assay. Discard any unused diluted antibody, do not store and reuse. Determine the number of wells needed. Samples and standards should be tested in duplicate. Allow 16 wells for the standard curve. Each well will be coated with 100uL of Coating Antibody Working Stock. Perform a 1:1870 dilution of GS0227 Lot A339 in 1xPBS to make the Coating Antibody Working Stock.

Detection Antibody GS0258 Lot#A374: Dilute the detection antibody just prior to use in the assay. Discard any unused diluted antibody, do not store and reuse. Perform a 1:1000 dilution of GS0258 Lot A374 in Sample Dilution Buffer (Section 6.) to make the Detection Antibody Working Stock.

IL6 Purified Protein Standard GS0177 Lot#A266: Dilute the 0.1 mg/mL IL6 purified protein standard just prior to use. Discard any unused diluted standard. Perform a 1:10,000 dilution of GS0177 LotA266 in Sample Dilution Buffer for the top standard concentration of 10ng/mL.

8. Procedure

8.1. Prepare Block Buffer, Sample Dilution Buffer, and Wash Buffer in advance. See Section 6., Reagent Preparation.

8.2. Dilute the Coating Antibody in 1xPBS according to the Lot Specific Instructions detailed in Section 7. Distribute 100uL of Coating Antibody Working Stock per well of a 96 well plate. Cover the plate and incubate at 4ºC overnight.

8.3. Proceed with the remaining ELISA steps the following day.

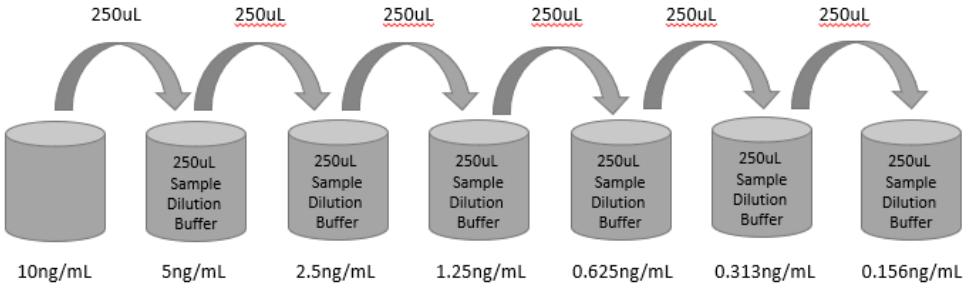
8.4. Wash the plate with Wash Buffer (Section 6) by aspirating each well and filling with wash buffer (300uL per well). Repeat twice more for a total of three washes. After the last wash remove any remaining wash buffer by aspiration or by inverting the plate on a clean paper towel.

8.5. Block the plate by adding 200uL per well of Block Buffer (Section 6) and incubating the plate at room temperature for one hour. Shaking is not required.

8.6. Prepare sample and standard dilutions while the plate is blocking.

8.6.1. Prepare samples by diluting in Sample Dilution Buffer, see Section 6.

8.6.2. Prepare at least 500uL of a top standard at 10ng/mL in Sample Dilution Buffer according to the Lot Specific Instructions found in Section 7. Perform 2x serial dilutions by removing 250uL of the top standard and dilution in 250uL of Sample Dilution Buffer. Repeat for a seven-point curve.



8.7. Repeat the wash steps as described in 8.4.

8.8. Add 100uL of samples and standards to the ELISA plate. Make sure to include a Sample Dilution Buffer only negative control. Incubate the samples and standards on the plate for one hour at room temperature. Shaking is not required.
8.9. Prepare the Detection Antibody Working Stock according to the Lot Specific Instructions in Section 7.
8.10. Repeat the wash steps as described in 8.4.
8.11. Add 100uL of Detection Antibody Working Stock per well and incubate the plate one hour at room temperature. Shaking is not required.
8.12. Prepare a Streptavidin-HRP solution in Sample Dilution Buffer according to the manufacturer instructions.
8.13. Repeat the wash steps as described in 8.4.
8.14. Add 100uL of Streptavidin-HRP solution per well and incubate the plate for 30 minutes. Protect the plate from exposure to light during this time.
8.15. Wash the plate a total of five times according to the instructions in step 8.4.
8.16. Add 100uL of TMB solution and incubate the plate at room temperature protected from light for 5-30 minutes. Optimal incubation times should be determined by the user.
8.17. Add 50uL of a stop solution (2N Sulfuric Acid) to each well.
8.18. Read the plate in a microplate reader at 450nm.
8.19. Calculate the Results
8.19.1. Calculate the average absorbance value for the blank negative control and subtract this value from the average absorbance values for all other samples and standards.
8.19.2. Create a standard curve by plotting the absorbance values for each standard concentration (y-axis) against the standard protein concentration (x-axis). Use graphing software to draw the best curve through these points to construct the standard curve. A four-parameter curve fit is recommended.
8.19.3. Determine the concentration of target protein in the sample by interpolating the absorbance value (background subtracted) against the standard curve. Multiply the resulting value by the appropriate sample dilution factor.
8.19.4. Samples with absorbance values above that of the top standard, or below the lowest standard should be reanalyzed with an appropriate adjustment of dilution.

Example Standard Curve.