Diaclone ELISA development

General Protocol

Note: This protocol is given as a general procedure to assist when using Diaclone Capture and Detection antibodies for ELISA testing. Optimal dilutions of all reagents, samples and controls as well as the incubation times should be determined by each laboratory for every application.

For research use only

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Reagents and Materials required but not supplied

- Coating Buffer: e.g. PBS
- Blocking Buffer: e.g. PBS with 5% BSA.
- Wash Buffer: e.g. PBS with 0.05% Tween
- Diluent for Sample and Standard: e.g. PBS 1%BSA
- Detection Solutions: e.g. Streptavidin-HRP and TMB
- Stop Solution: e.g. diluted H_2SO_4
- 96 well microtitre plates
- Plate covers/sealers
- 5 ml and 10 ml graduated pipettes
- 20 µl to 1,000 µl adjustable single channel micropipettes with disposable tips
- 50 µl to 300 µl adjustable multichannel micropipette with disposable tips
- Multichannel micropipette reservoir
- Beakers, flasks, cylinders necessary for preparation of reagents
- Device for delivery of wash solution (multichannel wash bottle or automatic wash system)
- Microtitre plate reader capable of reading at 450 nm (620 nm as optional reference wave length)
- Glass-distilled or deionised water

ELISA Plate Coating and Blocking

1: Dilute the capture antibody to the appropriate concentration (as per insert) using coating buffer (10ml final volume required per plate)
2: Add 100µl of the capture antibody solution to all wells of the plate, cover and incubate overnight at 4oC
3: Aspirate the contents of each well, then wash the plate at least twice using 400µl of wash buffer in each well. Following the final wash ensure all remaining buffer is removed by gentle blotting on absorbent paper
4: Add 250µl of blocking solution to all wells of the plate, cover and incubate at RT for 2 hours
5: Repeat step 3, the plate is now ready to run the assay

ELISA Assay Procedure

6: Add 100µl of prepared standard, sample and control dilutions to appropriate wells, cover the plate and incubate at RT for a minimum of 1 hour
7: Repeat step 3
8: Add 100µl of the diluted detection antibody (diluted as per insert) to every well, cover the plate and incubate at RT for a minimum of 1 hour
9: Repeat step 3
10: Add 100µl of the Streptavidin-HRP solution to all wells, cover the plate and incubate at RT for 30mins
11: Repeat step 3
12: Add 100µl of the TMB solution to all wells, cover the plate and incubate at RT for a minimum of 10mins
Note: Incubation time of the substrate solution is usually determined by the ELISA reader performance. Many ELISA readers only record absorbance up to 2.0 O.D. Therefore the colour development within individual microwells must be observed by the analyst, and the substrate reaction stopped before positive wells are no longer within recordable range
13: Add 100µl of stop solution to all wells and within 30mins measure the absorbance readings at 450nm (when available with 620nm as the reference wavelength, 610-650 is acceptable)