QED's GEM® (General ELISA Methodology) Kit provides all the reagents and supplies needed to custom design ELISA's for your applications. Each kit contains:

1. 6 96-well ELISA plates
2. 4 ml each 10X Coating Buffer Components A and B
3. 4 ml 10X Antibody Diluent
4. 200 ml 10X Wash Buffer
5. 0.5 ml Secondary Antibody-Horseradish Peroxidase Conjugate
6. 1 bottle Substrate Solution (ABTS)

Dilute 10X reagents with distilled H_2O for use. Store unused reagents at 4^oC.

Required but not provided:
Phosphate-buffered saline (PBS)
Bovine serum albumin (BSA)
Tween 20
Distilled H_2O

General ELISA Protocol

1. Antigen is bound to the wells of the ELISA plates in 50 ul 1X Coating Buffer. Prepare Coating Buffer by first preparing 1X solutions of Components A and B, then mix 3.5 parts A + 1.5 parts B, and dilute this mixture to 100 ml.

   We recommend testing a range of antigen concentrations from 5 ug/ml-200 ug/ml. Antigen-coated plates are sealed with plastic wrap and incubated overnight at room temperature.

2. The next day, plates are blocked for 30 minutes with 1% BSA-PBS-0.05% Tween 20. This solution is removed by inverting the plates, then serial dilutions of the first antibody in 1X Antibody Diluent are added (50 ul/well) for 30 minutes at room temperature with gentle agitation (such as on a rocker platform).

3. Plates are washed 3x with 1X Wash Buffer by filling all wells then inverting plates.

4. Secondary antibody, anti-Ig-horseradish peroxidase (HRP) conjugate, is diluted in 1X Antibody Diluent. The user should determine the optimal dilution for their secondary antibody. Diluted secondary antibody is added to each well (50 ul/well) for 30 minutes at room temperature with gentle agitation.

5. Plates are washed 3x with 1X Wash Buffer.

6. Each well receives 100 ul of substrate solution. Plates are incubated for 30 minutes at room temperature. Optical density (O.D.) readings are taken at dual wavelengths of 405 nm-490 nm or at single wavelength of 405 nm.

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