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. 40 ml Coating Buffer
3. 25 ml 10X Antibody Diluent/Blocking Solution*
4. 250 ml 10X Wash Buffer*
5. 0.1 ml Secondary Antibody-Horseradish Peroxidase Conjugate
6. 1 bottle Substrate Solution (ABTS)

Required but not provided: Distilled H_2O

General ELISA Protocol

1. Antigen is bound to the wells of the ELISA plates in 50 ul/well Coating Buffer.

   We recommend testing a range of antigen concentrations from 1 – 100 ug/ml. Antigen-coated plates are sealed with plastic wrap and incubated overnight at room temperature.

2. The next day, the coating buffer is removed by inverting the plates and the wells are blocked for 30 minutes with 200-300 ul/well of 1X Antibody Diluent/Blocking Solution, prepared by diluting the 10X solution in distilled H_2O. The blocking solution is removed by inverting the plates, then serial dilutions of the first antibody in 1X Antibody Diluent/Blocking Solution are added (50 ul/well) and incubated for 30 minutes at room temperature with gentle agitation (such as on a platform rocker).

3. Plates are washed 3x with 1X Wash Buffer (prepared by diluting the 10X buffer in distilled H_2O) by filling all wells then inverting the plates.

4. Secondary antibody, anti-Ig-horseradish peroxidase (HRP) conjugate, is diluted in 1X Antibody Diluent/Blocking Solution. 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, and blotted on absorbent towels to remove residual liquid.

6. Each well receives 100 ul of substrate solution. Plates are incubated for 10-30 minutes at room temperature. Optical density (O.D.) readings are taken at dual wavelengths of 405 nm-490 nm or at a single wavelength of 405 nm.

* 10X solutions may form crystals when refrigerated; warm slightly to re-dissolve or dilute entire bottle at one time.