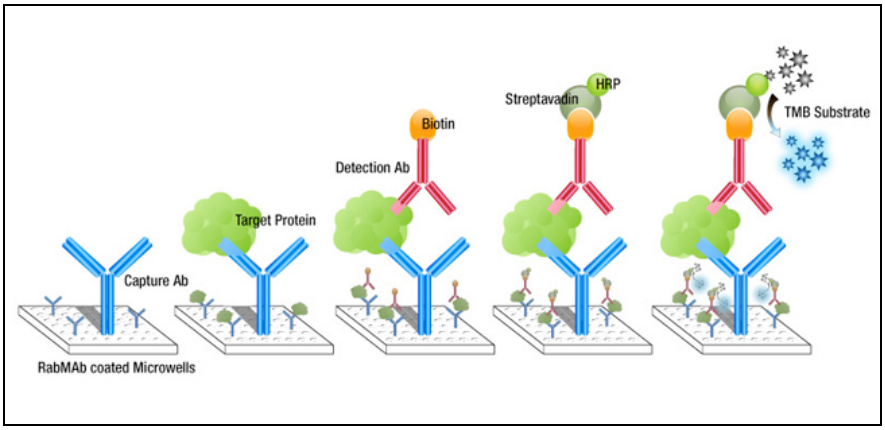
Enzyme Linked ImmunoSorbent Assay → **ELISA** is a tool used to detect and measure proteins, biomarkers, and small molecules (among countless other targets) using antibodies. There are many different formats for an ELISA; in this case the Streptococcus equi subsp. equi ELISA is in a sandwich format. In this format, there is a capture antibody and a detector antibody to detect and measure the Streptococcus "SeM" protein. See figure below:



Monoclonal antibody MAB212P coated on plate, Target protein is Streptococcus equi subsp equi bacteria, detection antibody is MAB211P-Biotin, tracer is Streptavidin HRP and TMB is the HRP enzyme substrate.

Terminology:

- Tween – Detergent used in the wash buffer
- ON@4°C – Overnight at 4 degrees Celsius
- RT – Room Temperature
- Capture – Antibody coated on the plate
- Detector – Antibody paired with the capture to detect the target (Strep equi)
- Biotin label – Small molecule conjugated to the tracer antibody, as anchor for detection
- SA – Streptavidin, this is what biotin binds at a very high affinity
- HRP – Horse Radish Peroxidase, this is the enzyme that reacts with the developer, and is conjugated to the SA
- Tracer – Streptavidin HRP, binding to the biotin on the tracer antibody
- TMB – Substrate that when exposed to HRP generates a blue product
- HCL – Hydrochloric acid, 0.6N, used to stop reaction of HRP and TMB, changing color to yellow
- Wash – plate is washed between steps, using an automated plate washer filled with PBS Tween 0.05%.

Materials:

- MAB211P-biotin (detector antibody)
- MAB212P (capture antibody)
- Nunc immunosorbent microtiter plates
- Streptavidin HRP (Jackson Immunoresearch)
- 0.15M PBS, pH 7.6, coating buffer (SOP attached)
- 1% Non Fat Dried Milk in PBS (blocking buffer)
- 0.15M PBS, 0.05% tween 20, wash buffer

- Strept-avidin HRP (binds biotin labeled antibodies)
- TMB (substrate)
- 0.6N HCL (stop solution)
- MAB211P-biotin detector, MAB212P – capture. (matched pair monoclonal antibodies)
- PlyC Lysin (recombinant phage lysin that disrupts the Streptococcus membrane expose the surface SeM protein).

Equipment:

- 12 channel pipet, pipet tips for 200 ul, 1000 ul.
- Single channel pipets, p20, p100, p1000 and accompanying tips
- Reagent boats
- ELISA Plate Washer
- ELISA plate reader (A450)
- Timer

Protocol:

ELISA Plate

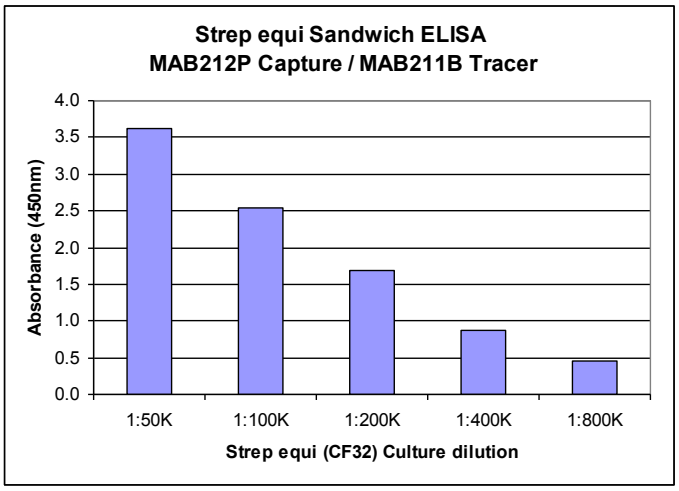
	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

1. Prepare a plate map for your samples and controls
2. Coating the plate:
    a. Prepare enough of MAB212P (capture antibody) to coat # of desired wells with 50 ul of MAB212P at 2 ug/ml in PBS.
        i. If stock antibody is at 1 mg / ml, this is a 1:500 dilution (1000 ug / 2 ug).
            1. To prepare 10 mls of coating antibody, pipet 20 ul of the antibody (at 1 mg/ml) to 9.98 ml of PBS, mix well / gently
            2. Pipet 50 ul of the antibody solution into each well using a multichannel pipet:
    b. Cover plate and incubate plate at 4C overnight
    c. Next day, wash plate 1X using automated plate washer (fill 300 ul)
3. Blocking:
    a. Prepare 1% NFDM in PBS
        i. 1 gram NFDM + 100 ml of PBS
        ii. Do not keep this reagent for more than 1 week at 4C.
    b. After capture antibody coating and washing, pipet 300 ul in each well using multi-channel.
        i. Incubate 1 hour at RT OR ON at 4C.

ii. Wash plate 1X post blocking.

4. Running the ELISA: Include + and – controls.
   a. Positive control, a sample from culture and (-) control, wells with NO antigen (strep equi)
   b. Prepare the sample:
      i. Strep equi CF32 culture (or your sample from water / compost) starting at 1:50K dilution treated with lysin at 10μg/mL in PBS (15min incubation at 37^0C prior to addition to plate).
         1. The starting dilution of your water / compost sample may be different, likely much more dilute than the culture we worked with. Start neat, and do 10 fold dilutions to see where you are.
      ii. Pipet 50 ul of prepared sample (and controls) into designated wells
      iii. Incubate for 30 minutes @ 37C or 1 hour at RT, with rotation if possible.
      iv. During this incubation , prepare a 0.5 ug/ml solution of the detector antibody MAB211P-biotin, in blocking buffer (1% NFDM)
      v. Wash plate 1X with automated plate washer, tap out residual liquid
      vi. Pipet 50 ul / well of the detector antibody (MAB211P-biotin)
      vii. Incubate@ 37C for 30 minutes or 1 hour at RT, with rotation if possible.
      viii. During this incubation, prepare a 1:10,000 dilution of the Streptavidin HRP in wash buffer (1 plate requires 5 mls)
         1. We typically have a 1:10 stock dilution and prepare a 1:1000 of that
            a. 5 ul (1:10) in 5 ml of wash buffer.
      ix. Wash plate 1X with automated plate washer, tap out residual liquid
      x. Pipet 50 ul of the Streptavidin HRP @ 1:10,000 and incubate the plate @ 37C for 30 minutes or 1 hour at RT, with rotation if possible.
      xi. Wash plate 4X, tap out residual liquid
      xii. Add 50 ul / well of the TMB, start timer for 10 minutes (cover plate)
      xiii. In 10 minutes, stop the enzyme reaction by adding 50 ul of 0.6N HCL
      xiv. Read the plate at A(450)

Typical Results using a CF32 culture provided by University of Kentucky:



SOP for the PBS used at MBS, recipe for 1 liter: Add chemicals to 900 mls, adjust pH and QS to 1 liter.

NaCl → 8 grams
KCL → 0.2 grams
KH_2PO_4 → 0.2 grams
Na_2HPO_4 → 1.15 grams

pH to 7.6 with either 6N HCL or 5N NaOH