Direct ELISA Protocol

Buffers and reagents

Antigen or antibody should be diluted in coating buffer to immobilize them to the wells: PBS pH 7.4. See Gibco recipe.

Blocking solution

Commonly used blocking agents are 1% BSA, serum, non-fat dry milk, casein, gelatin in PBS. – 1% BSA in TBS-T (tris- buffered saline with 0.05% Tween 20)

Rinse Solution

1X TBS → no tween

Wash solution

Usually PBS or Tris-buffered saline (pH 7.4) with detergent such as 0.05% (vol/vol) Tween20 (TBST).

Antibody dilution buffer

Primary and secondary antibody should be diluted in 1% BSA in TBS-tween blocking solution to reduce non-specific binding.

Antibody should be stored in 2-8 degrees C; see minifridge.

Stop Solution

4M HCL

Fisher:

- 96 well plates - we use Thermo Scientific Cat# 12-565-136
- Coating buffer- Gibco PBS pH 7.4 - Cat# 0-010-031

Sigma:

- Tween 20 - Cat# P7949-100mL
- BSA- Cat# A7906

Biolegend:

- Substrate solution- TMB substrate set Cat# 421101
- Alternative coating buffer - Sodium bicarbonate buffer pH 9.5 Cat# 421701

Biorad:

- Tris Buffered Saline- Cat# 1706435

General Procedure

<u>Coating antigen/analyte to microplate</u>

DAY 1

- Dilute each protein to a final concentration of 20 μg/ml and 1.25 μg/mL in 1X PBS. Make enough to cover as many wells as necessary. Coat the wells with the protein by pipetting 50 μl of the dilution in the each of the wells.
- Cover with a plate sealer and foil and incubate 4 degrees C overnight.

DAY 2

- Aspirate the coating solution by turning plate over and dumping the solution in the sink and tapping plate on top of tissues. **Rinse** each well TWCE with 200 μL of 1X TBS (no Tween). The solution is removed by flicking the plate over a sink. Aspirate or decant and tap the plate gently over a stack of tissue to remove excess liquid. – not necessary to change tips in this step, but do not touch the bottom of the wells.

<u>Blocking</u>

- Prepare enough 1% BSA in TBS-T for the respective wells to make the block solution and the antibody dilution.
- Add 200 μL of the **block solution** to each well with its respective block percentage – not necessary to change tips but do not touch the bottom of the wells.
- Cover with plate sealer and incubate at room temp (23 C) with gentle agitation (aka place in a shaker) for 1.5 hours.
  TIME IN: TIME OUT:
- Pick up from shaker and aspirate or decant and tap the plate gently to remove excess liquid in sink and then tap on top of tissues.

<u>Incubation with the antibody</u>

- Dilute 4G10 Platinum HRP conjugate (1:5000) in 1% BSA in TBS-T.
- Add 100 μL per well of the **antibody solution** – not necessary to change tips but do not touch the bottom of the wells.
- Cover with a plate sealer and incubate at 23C on shaker for 1.5 hours.
  TIME IN: TIME OUT:
- Pick up from shaker and dump the antibody solution in the sink and tap plate on tissues.
- **Wash** the wells 3 times with 200 μL per well of Wash Buffer (1X TBS-T). Once Wash Buffer has been added to each well, seal plate and place on shaker for 5 minutes. Take off shaker, dump wash buffer in sink, tap excess liquid out and repeat until the wells have been washed 3 times.

- Remove Wash Buffer after the third wash and **rinse** twice with 200µL per well of 1X TBS (no tween). Aspirate or decant and tap the plate gently over a stack of tissues to remove excess liquid.

Detection

- TMB substrate comes in Substrate A and Substrate B. Mix the two substrate solutions in a conical tube with a 1:1 ratio. Calculate 100 uL x # of wells (round up the nearest mL so you don't run out. THIS IS CRUCIAL.
- Dispense 100 µl of the **TMB substrate solution** per well with a multichannel pipet.
    - BE SURE TO CHANGE TIPS
    - MAKE SURE NO BUBBLES IN THE TIPS IN THIS STEP
    - ALSO BE FAST, AS COLOR WILL DEVELOP
- After substrate has been added to wells, place plate in a drawer so the color can develop in the dark
- After sufficient color development (if it is necessary) add 100 µl of stop solution to the wells
    - APPLY TO WELLS IN THE SAME ORDER THAT SUBSTRATE WAS APPLIED.
    - MUST CHANGE TIPS HERE.
- Read the absorbance (optical density) of each well with the Tecan Plate Reader.
    - 450 nm filter absorbance.
    - There is a file in the BIOMOD folder called ELISA.
    - Contingency if you can't find the folder: The only change is the type of plate we use and the filter absorbance.
- Note: some enzyme substrates are considered hazardous (potential carcinogens), therefore always handle with care and wear gloves. ☺