ELISA Protocols

**Reagents**

|  |  |  |
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| Reagent | Manufacture | Code |
| NUNC 96 well plate Maxisorp |  |  |
| Bicarbonate buffer |  |  |
| AD6 peptides: |  |  |
| PBS |  |  |
| Tween |  |  |
| FBS |  |  |
| Rabbit polyclonal antibody |  |  |
| Anti-rabbit HRP |  |  |
| TMB |  |  |
| Phosphoric acid |  |  |

**Procedure**

Antigen coating

* Dilute antigen of interest to the desired 1ug/mL in carbonate-bicarbonate buffer and add 100uL/well and leave overnight at 4oC.

Block plates

* Prepare blocking buffer 2% FBS in PBS
* Wash plate 4x (0.1% tween-20 in PBS)
* Add 150uL/well of blocking solution and incubate RT for 1h (min)

Add Polyclonal rabbit Ab

* Dilute 10uL/mL pAb in Blocking solution
* 1:10 serial dilution
* Wash plate 4x
* 100uL of pAb
* Incubate for 1 hr

Secondary

* Dilute antibody
* Wash 4x
* Add 100uL/well of secondary antibody anti-rabbit HRP
* Incubate for 1 hr at RT

Development buffer

* Wash 4x
* Add 100uL/well of TMB solution for 30mins max
* Stop reaction with 1M Phosphoric acid
* Read samples at 450nm

Locate reagents

* Coating buffer – Carbonate Bicarbonate
* Washing buffer – PBS + Tween 0.1% (1mL in 100mL of PBS)
* Blocking buffer – FBS + PBS
* Primary pAb – white box marked around A1 corner with E (currently at 0.47mg/mL) dilute in blocking buffer.
* Secondary goat anti-rabbit – ELISA Fridge dilute in blocking buffer
* TMB – ELISA Fridge
* Phosphoric acid – bench

How to make carbonate-bicarbonate

1. Prepare a 0.2-M solution of anhydrous sodium carbonate (2.2 g/100 mL).
2. Prepare a 0.2-M solution of sodium bicarbonate (1.68 g/100 mL).
3. Combine 4 mL of carbonate solution from Step 1 and 46 mL of bicarbonate solution from Step 2.
4. Bring to 200 mL with H2O. Final pH will be 9.2.