

Elisa protocol for one 96-well plate

to prepare:

5ml-50ug/ml BSA conjugated peptide in 10mM NaN_3 per well

50 ul 1 M NaN_3

5ml water

2.5 ul 10% BSA stock

70ml blotto

0.15 M NaCl 0.63 g

20 mM Tris-HCl, pH 8 1.4 ml of 1 M stock

4% nonfat dry milk 2.8 g

0.1% tween-20 70ul tween-20

50 ml-PBS

PBS 1 liter

0.15 M NaCl, 0.45g

10 mM phosphate, pH7 0.5 ml of 1 M stock

5.5 ml 1 step ultra TMB developing solution

5 ml-2 M H_2SO_4

450 ul- 5ug/ul K57/1

1.03 ul K57/1 2.2 ug/ul stock

449 ul Blotto

5ml-1/10000 KPL horseradish peroxidase in blotto

.5ul KPL horseradish peroxidase stock

5ml Blotto

buffers:

Notes:

Record times of all incubations and repeat as exactly as possible, Measure room temp.

Developing solution must be warmed to room temp before use.

Antibodies and peptide stocks should be kept cold.

Make notes specific to each ELISA on excel spreadsheet

Do not freeze horseradish peroxidase conjugated second antibody after use, refrigerate.

Protocol:

1)Coat plates

Add 30 µl of 50 µg/ml BSA control to the 8 wells in column 1. Add 30 µl of reused 50 µg/ml BSA conjugated peptide in 10 mM NaN₃ solution to all wells in columns 2-8. Allow samples to incubate in 96-well plates overnight at 4°C. Use or freeze plate within 24 hours.

2) Remove antigen liquid.

Samples are to be reused, remove using the multi-channel pipetman, return to original tube, label with # of uses, and store at -20°C. Do not use ANY azide (NaN₃) containing solutions after this point. Azide kills the HRP 2° antibody.

3) Block nonspecific protein binding sites.

Add 150 µl of Blotto +0.1% tween-20 per well and rock at room temp for 45 min.

4) 1° antibody incubation.

Remove blocking solution, dump and blot dry. Add 30 µl tween blotto to columns 3-12. Add antibody dilutions: 30 µl per well in column 1 and 30 µl in column 2 and 30 µl to the 30 µl blotto in column 3. Serially dilute by 50% for each successive row, by pipetting three times to mix, removing 30 µl and adding to next row to right. Tips can be rinsed in blotto in between dilutions if there is any solution that gets stuck in them. Repeat until row 11 then remove 30 µl and dispose.

Row 12 should have no antibody ever.

Rock for 45 min at room temp.

5) Remove antibody dilutions. To wash, add 150 µl of Blotto per well. Rock for 5 min at room temp. Remove wash solution by dumping. Repeat washes two times.

6) After last wash, add 50 µl of KPL horseradish peroxidase (KPL HRP GxM H&L) conjugated second antibody, diluted in blotto. Rock for 45 min at room temp.

7) Remove HRP-conjugated solution. To wash add ≥150 µl of PBS (0.15 M NaCl, 10 mM phosphate, pH7) per well. Rock for 5 min at room temp. Remove wash solution by dumping. Repeat washes two times.

8) To develop, add 50 µl of developing solution per well. Allow plates to develop on rocker at room temp ~5 minutes until sufficient blue color develops then stop developing with 50 µl 2 M H₂SO₄

9) Read Abs₄₅₀ on Hell plate reader, record final values in excel spreadsheet