**ME191: Ms IgM assay**

**Plate layout and sample key**

Plate layout is big so I’ve attached it on another sheet.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Mouse #** | **Genotype** | **Treatment** |
| 1 | 412S | C57Bl/6J | 7d DMEM |
| 2 | 343S | MMP3 -/- | 7d DMEM |
| 3 | 408S | C57Bl/6J | 7d H1N1 |
| 4 | 409S | C57Bl/6J | 7d H1N1 |
| 5 | 346S | MMP3 -/- | 7d H1N1 |
| 6 | 422S | MMP3 -/- | 7d H1N1 |

Prepare reagents (day 1 rgts in yellow, day 2 not highlighted)

* Coating buffer: 0.05M carbonate-bicarbonate, pH 9.6
* Wash solution: 50mM Tris, 0.14M NaCl, 0.05% Tween-20, pH 8.0
* Blocking buffer/sample/conjugate diluent: TBS + 1% BSA
* Enzyme Substrate: TMB (in box in RH 4C)
* Stop: 0.18M H2SO4

Prepare standards

Old standards:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Standard** | **ng/mL** | **volume diluent** | **volume serum** | **source serum** |
| 1 | 1000 | 1 mL | 2 uL | stock |
| 2 | 500 | 500uL | 500uL | std1 |
| 3 | 250 | 500uL | 500uL | std2 |
| 4 | 125 | 500uL | 500uL | std3 |
| 5 | 62.5 | 500uL | 500uL | std4 |
| 6 | 31.25 | 500uL | 500uL | std5 |
| 7 | 15.625 | 500uL | 500uL | std6 |
| 8 | 0 | 500uL | NA | NA |

Kit standards:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Standard** | **ng/mL** | **volume diluent** | **volume standard** | **source standard** |
| 1 | 1000.00 | 0 | 1000 | stock |
| 2 | 333.00 | 333 | 150 | std1 |
| 3 | 111.00 | 333 | 150 | std2 |
| 4 | 37.00 | 333 | 150 | std3 |
| 5 | 12.33 | 333 | 150 | std4 |
| 6 | 4.11 | 333 | 150 | std5 |
| 7 | 1.37 | 333 | 150 | std6 |
| 8 | 0.00 | 333 | 0 | NA |

I did it this way (rather than the same values as the “old” set) because the kit provides 2000ng standard total, of which 1000 had already been used.

Prepare coating antibody dilution

1. Pipet 30uL coating antibody into 15mL conical tube
2. Pipet 3mL coating buffer into 15mL conical.

Plate 1 day 1

Allow 2.5h

1. Add 100uL diluted coating antibody to each well of a strip well plate.
2. Incubate RT 1h.
3. Wash plate 2x with wash buffer. Aspirate final wash.
4. Add 300uL blocking buffer to each well.
5. Incubate RT 30min.
6. Wash plate 2x with wash buffer. Aspirate final wash.
7. Add 100uL standards/samples to each well.
8. Incubate O/N 4C.

Day 2

1. Aspirate wells.
2. Wash plate 2x with wash buffer. Aspirate final wash.
3. Add 100uL diluted HRP detection antibody to each well.
4. Incubate RT 1h in a drawer.
5. Just before end of incubation, pipet 2.5mL of TMB and solution B (both in box together) into a conical tube.
6. Wash plate 5x with wash buffer. Aspirate final wash.
7. Add 100uL TMB substrate solution to each well.
8. Develop plate 2min.
9. Add 50uL stop solution per well.
10. Read plate at 450nm with reference wavelength.