**LM030: testing mouse IgM ELISA standards**

We have two mouse IgM standards. I need to test which standard works best with the coating antibody.

**Plate layout**

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

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 (optimized by Kelly; in RH 4C)
* Enzyme Substrate: TMB (in box in RH 4C)
* Stop: 0.18M H2SO4

Prepare standards

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Standard** | **ng/mL** | **volume diluent** | **volume antibody** | **source antibody** |
| 1 | 1000 | 2 mL | 4 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 |

Prepare coating antibody dilution

1. Pipet 50uL coating antibody into 15mL conical tubes.
2. Pipet 4950uL coating buffer into 15mL conical.

Plate 1 day 1 (20231129)

Allow 2.5h

1. Add 100uL diluted coating antibody to each well.
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 (20231130)

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. Wash plate 5x with wash buffer. Aspirate final wash.
6. Add 100uL TMB substrate solution to each well.
7. Develop plate 2min.
8. Add 50uL stop solution per well.
9. Read plate at 450nm with reference wavelength.