

**Stamatatos Lab**  
**TZM-bl Neutralization protocol**

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1. Plate 5,000 TZM-bl cells in 50ul of complete DMEM per well in a 96 well flat-bottom plate
2. Incubate O/N at 37°C
3. Next day, serial dilute Mab or Serum in 30ul/well of complete DMEM in a 96 well U-bottom plate
4. Then add 30µl of diluted virus to each well.
5. Incubate at 37°C for 1.5 hours.
6. During the last 45 min of incubation, add 50ul of polybrene (4ug/mL) to the TZM cells and incubate for 30 min at 37°C.
7. Aspirate polybrene DMEM from cells and replace with 50uL of virus + Mab/serum mixture.
8. Incubate at 37°C for 72 hours.
9. Aspirate the media from the plate and replace with 100uL of Luciferase Reagent, lyse for 15min at RT then read RLU on a luminometer.