Heparin Binding Assay – Biotinylated-FGF2

Materials

96-well high binding plate (Corning, 9018)
90% saturated (3.7 M) ammonium sulfate
Biotinylated-FGF2 (Shenandoah, 100-18B) *(see note below)*
Streptavidin-HRP (JacksonImmunoResearch, 016-030-084) *(see note below)*
1-step Turbo TMB-ELISA substrate solution (34022, Pierce)
PBS
PBST (0.2% Tween)
BSA (Sigma)
1 M H_2SO_4

Procedure

1. Dilute heparan sulfate in PBS as needed to get the desired amount in the well in 10 uL. The well is typically saturated with 10-30 ng of heparan sulfate.

2. Add 200 uL of 90% saturated ammonium sulfate to each well. Incubate overnight at room temperature.

3. Wash wells with 6 x 200uL of PBST. Remove all liquid from wells by hitting plates against paper towels.

4. Add 200 µl of PBST + 1% BSA to block wells. Incubate 1 h at 37°C. Remove all liquid from wells by hitting plates against paper towels.

5. Add 50 µl of biotin-FGF2 in PBST + 1% BSA. Incubate for 1 hour at room temperature. Wash 3 times with 200 µl PBST.

6. Add 50 µl of 1:5000 dilution of Streptavidin-HRP in PBST + 1% BSA. Incubate for 30 minutes at room temperature. Wash 5 times with 200 µl PBST.

7. Warm ELISA substrate to RT. Prepare enough for 100 µl in each well.

8. Add 100 µl of HRP substrate to each well. Develop at room temperature.

9. Stop the reaction with the addition of 100 µl of 1 M H_2SO_4 and read plate at 450 nm.

Notes

- 1:100 dilution of Biotinylated-FGF2 gives ~15 nM.
- Many reagents are already diluted 1:1 in glycerol for storage at -20 deg C. Adjust dilution for the assay accordingly.