**Anti FcRn ELISA Protocol**

1) Coat plate with IG3 diluted 1/1000 (use the 12/1/95 Ab) into PBS. 100 µl/well, Incubate overnight at 4 C.

2) Wash three times with PBS/0.05% Tween pH 7-7.6 (Take excess out by taping the plate against a paper towel).

3) Incubate supernatants, 200 µl/well for 1 hour @ 37 C.

4) Wash three times with PBS/0.05% Tween pH 7.0-7.6.

5) Make PBS/3%BSA ~pH 7.5

Dilute anti-β2m (polyclonal rabbit a human β2m) into this at 1/1000 dilution. Plate 100 µl and incubate 1 hour @37 C.

6) Wash three times with PBS/0.05% Tween pH 7.0-7.6.

7) Dilute peroxidase conjugate α-rabbit 1/5000 into PBS/3% BSA, plate 50 µl/well. Incubate plates 1 hour @ 37 C.

7) Wash three times with PBS/0.05% Tween pH 7.0-7.6.

8) Prepare a solution containing:

10 mg OPD (ortho phenylene diamine) in
*25 mL of citrate/phosphate buffer pH 5.5
add 12.5 µl of 30% $H_2O_2$.

* To prepare Citrate/Phosphate buffer:

20 mL 1M dibasic Sodium Phosphate (pH to 5.5 w/ 1M citric Acid)
170mL distilled water
200mL Final Volume

9) Add 200 µl/well to ELISA plates

10) Watch for color development (yellow-->brown, when the solution is yellowish/brownish)

-Quench rapidly with 50 µl of 3M sulfuric acid.
-Read absorbance @ 490 nm.