Protocol for analysis and/or sorting of FcRn expressing cells using receptor binding to IgG at pH 6.0. Note there are differences in this protocol and protocols for staining cells using mAbs directed against FcRn.

500 ml of 10x HBSS
6.3 μl $CaCl_2$ (1M)
2.5 μl $MgCl_2$ (1M)

KCl	2g
KH_2PO_4	0.3g
$MgSO_4$	0.5g
NaCl	40 g
Na_2HPO_4	0.45 g

All buffers kept cold and cells on ice throughout procedure (except when cells being detached)

Binding buffer: 10 mM Hepes, 0.25% BSA, 1x HBSS, pH 6.0 or 8.0 (pH using HCl or NaOH)

Use one confluent plate of cells. Detach cells with 1x PBS containing 4 mM EDTA. Mechanically detach cells using binding buffer (at appropriate pH) by pipetting in and out.
Count cells.
Put 1x 10^6 to 1 x 10^7 cells in one tube. Pellet 1500 rpm 5 min. Aspirate supernatant. Wash cells with buffer at appropriate pH. Pellet again and aspirate supernatant.
Resuspend cells in 200 - 500 μl of 50 μg/ml FITC-Fc.
Incubate on ice for 3-4 hours.
Add 2-3 ml binding buffer (at appropriate pH) to conical glass tube. Very slowly, add 0.5 ml FCS pH'd to either pH 6.0 or 8.0 (pH FCS with 1M HCl or 1M NaOH) to **bottom** of conical tube using a glass pasteur pipette. Should see layer of FCS on bottom.
Spin cells 1500 rpm, 5 min.
Aspirate to remove FCS and binding buffer without touching cells.
Resuspend in 0.5 ml cold binding buffer at pH 6.0 or 8.0. (Note the cells should not be washed because binding of Fc to FcRn is weak)
Filter cells through nylon mesh.