



FcRn/antibody interactions

An Immunitrack/GE collaboration

Sune Justesen, Immunitrack and Åsa Frostell, GE Healthcare

Sep, 2015

Imagination at work

GE Proprietary Information—Class III (Confidential)
Export Controlled—U.S. Government approval is required
prior to export from the U.S., re-export from a third
country, or release to a foreign national wherever located.

Biacore™ T200 assay conditions for studies of FcRn antibody interactions

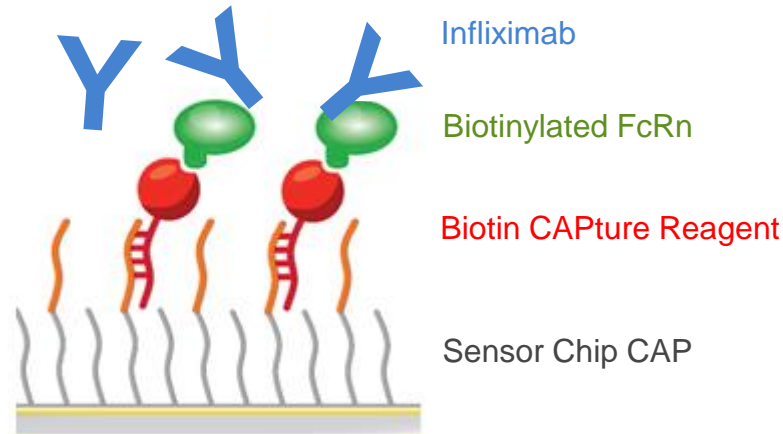


Fig 1. Assay principle. Using Biotin CAP Kit (GE Healthcare) a streptavidin conjugate is first bound to the chip followed by capture of biotinylated FcRn and binding of the antibody. At the end of the analysis cycle the surface is regenerated and ready for next determination. Running buffer: 20 mM phosphate, 150 mM NaCl, 0.05 % Surfactant P20, pH 6.0. The Biotin CAP kit contains Sensor Chip CAP, streptavidin conjugate and regeneration solution.

Antibody binding profiles for different species of FcRn at pH 6.0

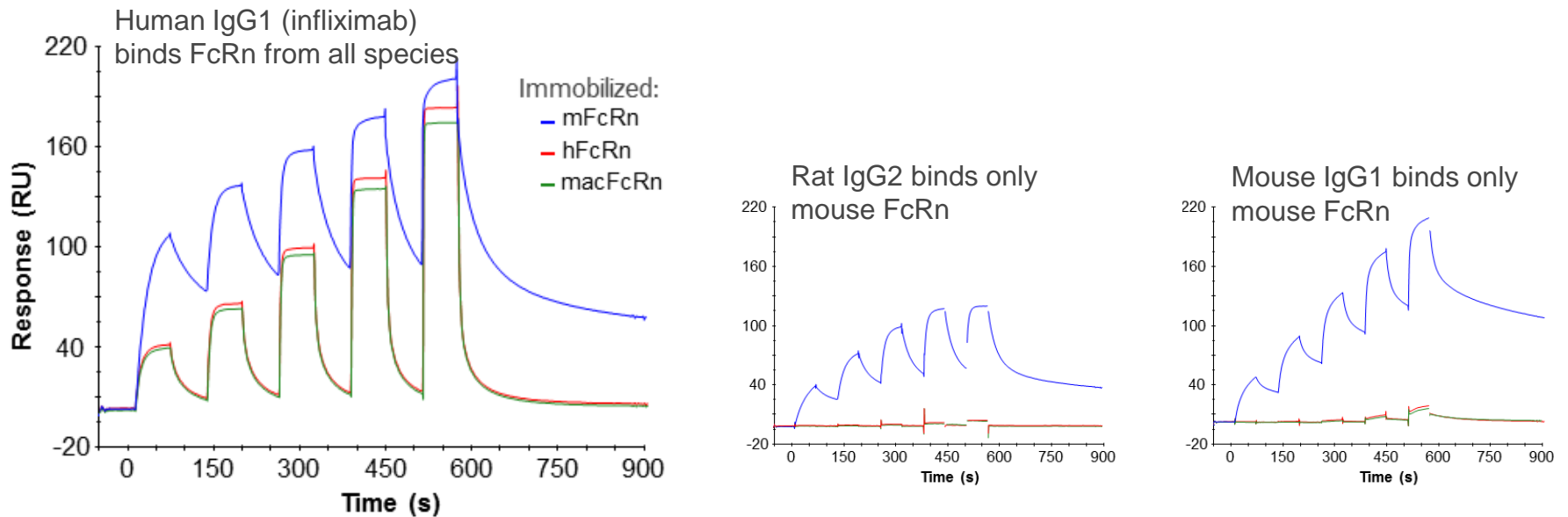


Fig 2. Examples showing binding profiles for different types of antibodies to human, macaque and mouse FcRn. Binding behavior towards human and macaque FcRn was very similar. All antibodies showed highest binding towards mouse FcRn. Binding specificities for all antibodies were in agreement with published data*. Biotin CAP Kit (GE Healthcare) was used with FcRn capture levels 90-100 RU and antibody concentration series: 25, 74, 222, 666 and 2000 nM. Running buffer: 20 mM phosphate, 150 mM NaCl, 0.05 % Surfactant P20, pH 6.0.

*Abdiche, Y. N. et al. 2014, mAbs, 7, 331-338.



pH dependent binding of antibodies to FcRn facilitated using Dual inject

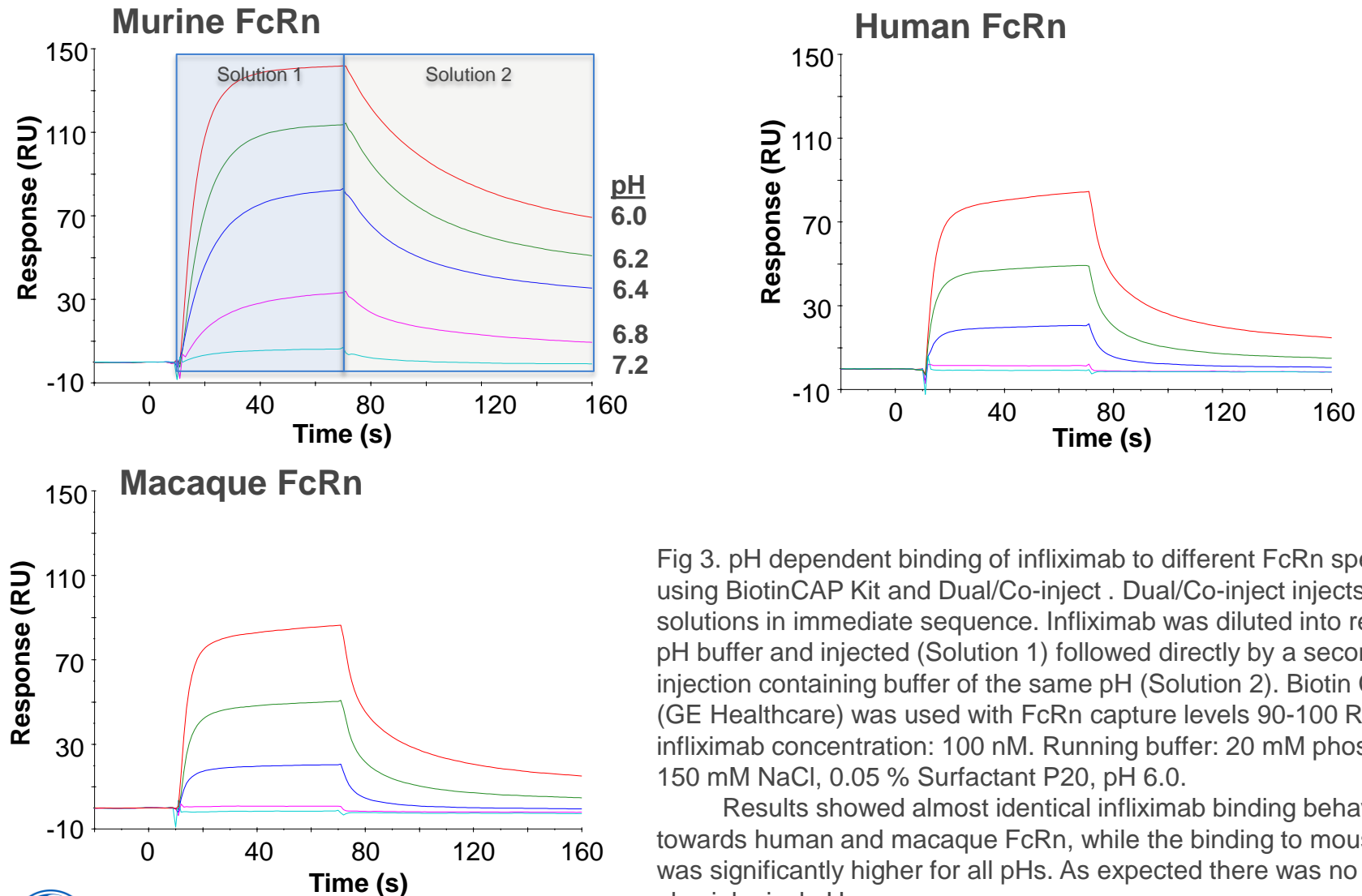


Fig 3. pH dependent binding of infliximab to different FcRn species using BiotinCAP Kit and Dual/Co-inject . Dual/Co-inject injects two solutions in immediate sequence. Infliximab was diluted into respective pH buffer and injected (Solution 1) followed directly by a second injection containing buffer of the same pH (Solution 2). Biotin CAP Kit (GE Healthcare) was used with FcRn capture levels 90-100 RU and infliximab concentration: 100 nM. Running buffer: 20 mM phosphate, 150 mM NaCl, 0.05 % Surfactant P20, pH 6.0.

Results showed almost identical infliximab binding behavior towards human and macaque FcRn, while the binding to mouse FcRn was significantly higher for all pHs. As expected there was no binding at physiological pH.



www.gelifesciences.com

GE, imagination at work, GE Monogram, Biacore are trademarks of General Electric Company.

All other third party trademarks are the property of their respective owners.

© 2015 General Electric Company. First published September 2015.

All goods and services are sold subject to the terms and conditions of sale of the company within GE Healthcare which supplies them. A copy of these terms and conditions is available on request. Contact your local GE Healthcare representative for the most current information.

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire, HP7 9NA
UK

