

Analysis of Intestinal Permeability in Mice

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Summary of Q&A (**last updated on 9/19/2016**)

During the experiment

Q#1. *Do you starve the mice including also food overnight in addition to water starve? And after the oral gavage, when you are waiting for 4 hours, do you put the water bottles back?*

A. We only water starve the mice as also mentioned in the protocol. In my recent experience, 5-6 h of water starvation also reduces variability between mice. During the 4h waiting time we put the water bottle back.

Q#2. *Should I use two standard curves respectively to determine the concentration of FITC if I want to analysis the gut permeability of wild type mice and IL6-/- mice?*

A. You can use only one standard curve and serum from one of the untreated WT mice of same genetic background (better littermate) to determine the concentration of FITC-dextran in the serum samples from both WT and IL6 KO mice.

Q#3. *How long can I keep the serum samples at 4 °C until the spectrophotofluorometer determination? And can I store the serum samples at -20°C or -80°C until I collect enough samples for spectrophoto determination?*

A. We kept samples at 4 °C for maximum of 2h after taking it out from mice and immediately analyzed for FITC determination by spectrophotometer. We always used fresh serum to analyze FITC concentration in serums but in theory if you protect from light and samples are nicely and similarly stored at -20°C, you should be able to detect FITC with minimum loss of its Fluorescence.

Q#4. *After oral gavage of FITC-dextran for 4 h, can I collect serum sample but not kill the mice, keep the mice for somedays and gavage FITC-dextran again and collect serum sample?*

A. You do not need to kill mice after 4h if you are able to take around 250 µl blood to get at least 100 µl serum.