**pH measurement of and sample collection from the gastrointestinal content in mice.**

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| **Materials**   * Test animals, 56 in total, 7 weeks old  |  |  |  |  | | --- | --- | --- | --- | |  | **Janvier** | **Taconic** | **Internally** | | **BALB/c** |  |  |  | | Males | 4 | 4 |  | | Females | 4 | 4 |  | | **C57BL/6JRj** |  |  |  | | Males | 4 | 4 |  | | Females | 4 | 4 |  | | **NMRI** |  |  |  | | Males | 4 | 4 |  | | Females | 4 | 4 |  | | **BALB/c germ free** |  |  |  | | Males |  |  | 4 | | Female |  |  | 4 | | **BALB/c streptomycin treated** |  |  |  | | Males |  | 4 |  | | Female |  | 4 |  | | **Total** | **24** | **32** | **8** |  * pH calibration buffers * pH-meter Brand and type: Consort, C3040 6 channel pH/Ion/conductivity/DO/ISE meter * pH-electrode Brand and type: \*\* MiniTrode, Part/ref: 238100 * Dissection equipment * Sterile Eppendorf tubes, 2 mL and 5 mL * Sterile SPO buffer, pH=6.4 * Precision scale * Vortex mixer * 1000 μL pipette * 1000 μL pipette tips * Timer * 70 % ethanol * Sterile cryo tubes * Sterile PBS with 20% glycerol, pH= 7.0 * Centrifuge, Sigma, 1-14K, Buch and Holm. * Sterile falcon tubes * Anesthetics: hypnorm/dormicum   \* <https://consort.be/wp-content/uploads/kataloog-20-06-online-1.pdf>  \*\* <https://www.hamiltoncompany.com/process-analytics/sensors/238100> |

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| **Different sections of the mouse GIT to be measured**   * Stomach * Duodenum *≈* 4 cm * Jejunum *≈* everything between duodenum and ileum * Ileum *≈* 2 cm * Caecum * The colon is bisected at its midpoint to separate the proximal and distal colon. |

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| **Preparations**  After transportation from the vendor, the mice are housed for 5-6 days. Female mice are handled on day 5, while male mice are handled on day 6.  Calibrate the pH electrode.   * Have marked sterile Eppendorf tubes/Falcon tubes\* ready, 7 for each mouse. Note the weight of the tubes. * Have marked cryo tubes ready, 7 for each mouse. * Have marked RNA tubes ready, 2 for each mouse.   The mice should not be in a fasted state for this experiment; therefore, it is preferred to start the experiment in the morning.  \* Note: The GIT content from the duodenum, ileum, proximal colon, and distal colon are transferred to Eppendorf tubes for further dilution in 1:10 SPO buffer. However, the content of the stomach, jejunum, and cecum exceeds the maximum capacity of an Eppendorf tube. Instead, a Falcon tubes are used. |

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| **Protocol**  The mouse is anesthetized, and the blood sample is drawn and stored in an Eppendorf tube at 5°C until further processing. After collection of the blood sample the mouse is euthanized, and dissection is performed:   1. The GIT from the stomach to the anus is removed from the mouse. 2. Starting from the stomach, the content is collected from each section and transferred directly to the Eppendorf tube.    1. The stomach is cut open and the content is scraped out into the tube.    2. Cut off the remaining sections of the GIT and squeeze the content into the tube.    3. Furthermore, collect tissue samples. One from the ilium and one from the colon.       1. A 1 cm segment of the ileum is excised, specifically from the region immediately preceding the cecum.       2. A 1 cm segment of the colon is excised specifically 2-3 cm after the cecum       3. Tissue samples are transferred to marked tubes containing RNA-later and stored at 5°C. 3. Weigh the tube with content. Then calculate the actual weight of the sample. 4. The ratio between the sample and buffer is 1:9 respectively. (90uL buffer to 10mg sample) 5. Homogenize the samples by vortexing. Some samples might need to be crushed, using a inoculation stick. 6. Measure the pH immediately after vortexing. Wipe the pH-electrode with 70% ethanol before each measurement. \*\*\*\* 7. Spin down the content of the Eppendorf tube (10 min, 13000 g) and discard the supernatant. 8. Add 500 uL PBS with 20% glycerol to the sample and vortex. 9. The samples are moved directly to a minus 80°C freezer   The procedure is performed for one mouse at a time and is repeated until blood, colon tissue, and GIT pH data from all mice are collected.  \*\*\*\*Collection of cecum content for FMT   1. After pH measurement of the cecum content in buffer, a 500 uL of fecal suspension is transferred to an Eppendorf tube, and steps 7, 8 and 9 are performed. 2. The rest of the fecal slurry is further diluted in 1:1 PBS-buffer with 20% glycerol. 3. The samples are moved directly to a minus 80°C freezer   Processing of blood samples   1. Blood samples are centrifuged for 8 min at 8000 g. Blood samples should be refrigerated as a minimum of 30 minutes prior to centrifugation. 2. The supernatant is transferred to a new Eppendorf tube which is then moved to -80°C. 3. The tissue samples are moved to a -80°C. |