**Buhlmann Fecal Calprotectin ELISA**

Alm Lab

Last updated by MM on 3/27/17

**MATERIALS AND EQUIPMENT**

* Buhlmann Fecal calprotectin ELISA 96-well format kit
* Biosafety cabinet
* 96-well plate reader (450 nm wavelength)
* Scale with mg resolution
* Small vortex inside biosafety cabinet
* Benchtop vortex with vortex adapter for 2 ml Eppendorf tubes
* Microcentrifuge for 2 ml Eppendorf tubes
* 15-ml conical tubes
* Disposable inoculation loops
* 2-ml Eppendorf tubes
* Benchtop plate rotator (450 rpm speed)
* 96-well plastic plate covers
* 96-well aluminum plate covers
* Multi-channel pipette and tips (100 uL – 300 uL range)
* Plastic reservoirs
* Paper towels

**PREPARE SAMPLES AND REAGENTS**

1. Thaw stool samples on ice for at least 2.5 hours. If stool samples are stored in RNAlater and will also be sequenced: Wash stool samples with PBS twice. Keep two 100 – 200 mg aliquots of stool for DNA sequencing in their respective tubes.

Note: Keep the number of freeze-thaw cycles consistent throughout a study. The more cycles you have, the more human cells are broken, and the higher fecal calprotectin levels you will measure.

1. Bring ELISA kit reagents to room temperature before starting, EXCEPT the 96-well plate and tubes with standards, high control and low control. Those stay at 4 degrees until you use them.
2. Draw plate map. Determine how many strips of the 96-well you will need.

Note: Each sample and standard must be measured in duplicate. Also reserve three wells for the low control, high control and blank.

1. Prepare Wash Buffer 1X if necessary. It comes in 10X format in a new kit.

**STOOL SAMPLE EXTRACTION**

1. Label and weigh empty 15-ml conical tubes together with a disposable inoculation loop. Record weights in a paper sheet.
2. Take out 0.05 to 0.1 g of stool sample (50 to 100 mg) by means of the inoculation loop and place it into the pre-weighted tube.
3. Weigh tubes containing stool samples. Record weights in a paper sheet.
4. Calculate grams of stool by subtracting weighs for each tube pre- and post-stool.
5. Add **Extraction Buffer** according to the formula:

*x* gstool \* 49,000 = *y* uLextraction buffer

For example: If you weigh 0.05 g stool, add 2450 uL of Extraction Buffer.

1. Swirl the inoculation loop to wash off any stool and discard it. Homogenize sample by vortexing for 30 seconds. Transfer 2 ml of sample to an Eppendorf tube.

Note: This is the only modification to the manufacturer’s protocol. We transfer 2 ml to an Eppendorf tube and then vortex for 30 minutes because we don’t have a vortex-adapter for 15 ml conical tubes.

1. Secure tubes to vortex adapter and vortex at maximum speed for 30 minutes.
2. Centrifuge tubes at 3000xg for 5 minutes.
3. Transfer the supernatant into a fresh, labeled tube.

Note: Extracts are stable for 6 days at 2-8 degrees Celsius, or for 18 months at -20C. It could be a good idea to break here and continue with dilution and assay the next day. However, any storage condition must be kept consistent throughout a study.

**SAMPLE DILUTION**

**HEALTHY** individuals samples:

1. Dilute stool extracts 1:50 with **Incubation Buffer** and mix well. For example:

*20 uL extract + 980 uL incubation buffer*

1. Let the samples equilibrate for at least 5 minutes at room temperature.

**IBD** patients samples:

1. Dilute stool extracts 1:150 with **Incubation Buffer** and mix well. For example:

10 uL extract + 1490 uL incubation buffer (pipette 745 uL twice)

1. Let the samples equilibrate for at least 5 minutes at room temperature.

**ELISA ASSAY**

1. Prepare a plate with sufficient strips to test the required number of standards, controls and diluted samples. Remove excess strips from the holder and re-seal them in the foil pouch together with the desiccant packs without delay. Store refrigerated.

Note: Each sample and standard must be measured in duplicate. Also reserve three wells for the low control, high control and blank.

1. Wash wells twice with 300 uL of **Wash Buffer** per well. Wait for **20 seconds**. Empty wells at the sink and tap plate firmly onto paper towels.
2. Pipet 100 uL of Standards A-E, low control, high control and blank in their respective wells.
3. Vortex each diluted sample for 5 seconds and pipet 100 uL into their respective wells.
4. Cover the plate with a plate sealer and incubate for 30 minutes (+ 5 min max) on a plate rotator set at 450 rpm at room temperature.
5. Carefully remove and discard the plate sealer. Empty wells at the sink and tap plate firmly onto paper towels.
6. Wash three times with 300 uL **of Wash Buffer** per well. Wait for **20 seconds** for each wash. Empty wells at the sink and tap plate firmly onto paper towels.
7. Pipet 100 uL of **Enzyme Label** to each well.
8. Cover the plate with a plate sealer and incubate for 30 (+ 5 min max) on a plate rotator set at 450 rpm at room temperature.
9. Carefully remove and discard the plate sealer. Empty wells at the sink and tap plate firmly onto paper towels.
10. Wash five times with 300 uL of **Wash Buffer** per well. Wait for **20 seconds** for each wash. Empty wells at the sink and tap plate firmly onto paper towels.
11. Pipet 100 uL of the **TMB Substrate Solution** to all wells.
12. Cover the plate with an aluminum plate sealer. Protect the plate from direct light and incubate for 15 min (+ 2 min max) on a plate rotator set at 450 rpm at room temperature.
13. Pipet 100 uL of **Stop Solution** to all wells. Remove air bubbles with a pipette tip.
14. Read absorbance at 450 nm within 30 minutes. Export CVS file from Excel and save in a USB stick. Name file in the following format:

Date\_Study\_Initials.cvs

For example: 170327\_FlagshipLongitudinal\_TP.xls would correspond to a file from the longitudinal Flagship study run on March 27th, 2017 by Tsultrim Palden.