IMMUNOLOGIC ASSAY

Aim: The goal is to determine whether our immunoassay is effective. We want to ensure that our antibodies are well able to detect the presence or absence of viral proteins from Chikungunya and Yellow Fever viruses. We took care to handle the proteins, not the infectious viruses.

Materials:
- PVDF or nitrocellulose membrane
- YFV E protein, CHIKV E2 protein*
- 4G2 antibody*
- Secondary antibody (anti-mouse alexafluor 488, Licor)*
- BSA (Sigma)
- PBS*
- Tween*
- Milk 5% (Regilait) in PBS-Tween*
- EZ-link kit
- Rocker agitator

Protocol:

Sample preparation:

The sample is tagged using EZ-Link kit (Pierce).

1. Add 500 ml of ultrapure water to the dry-blend Phosphate Buffered Saline (PBS).
2. Prepare 1 mg of antibody in 1.0 ml of PBS(+/-BSA1%).
3. Reconstitute 1 mg of lyophilized EZ-Link Plus Activated Peroxidase with 100 µl of ultrapure water and add it to the antibody solution or add the protein sample directly to the lyophilized activator.
4. In a fume hood, immediately add 10 µl of sodium cyanoborohydride to the reaction and incubate for 1 hour at room temperature.
5. Add 20 µl of Quenching Buffer and react at room temperature for 15 minutes.

→ *The conjugate can be stored at 4°C for up to 4 weeks.*

Membrane:

1. **Coating:** put 1 µl of a non-diluted antibody on the membrane, let the membrane dry for 5 minutes at room temperature.

2. **Saturation:**

• Put the membrane in PBS-Tween 0.5%-milk 5% on a rocker, 1 hour at room temperature.
• Replace the washing solution with a fresh one.

3. **Binding:** Add the sample in the PBS-Tween 0.5%-milk 5% solution at the appropriate dilution, incubate overnight at 4°C on a rocker.

4. **Washing:** Wash the membrane 3 times for 5 minutes on a rocker at room temperature.

5. **Revelation:** Reveal the membrane using approximately 1 ml of Pierce ECL Western Blotting substrate.

myECL imager (Thermofisher)

Generious gift from the CIBU of Institut Pasteur*