Version 2, June 6th 2017, by S. Bol, approved by Dr. Bunnik

Human IgG ELISA (Enzyme-Linked Immunosorbent Assay)

Used to quantify levels of human IgG in a solution using antibodies

Changes from version 1 to 2:

- Using Non-Animal Protein (NAP) buffer instead of FBS
- Clarified washing steps
- Lower amount of human IgG protein for standard curve and three-fold dilution

Materials & chemicals needed:

- 96 wells plate suitable for ELISA/EIA, clear, flat bottom, with lid, polystyrene, high bind (Corning Cat. # 3361)
- Non-sterile plastic reservoirs
- 8 or 12 channel pipette
- TMB solution (stored at 4°C)
- Non-Animal Protein buffer (stored at 4°C; keep sterile)
- PBS (stored at RT)
- Stopping solution (0.18 M H_2SO_4 (10 ml H_2SO_4 + 990 ml Milli-Q water))

For the detection of **human IgG protein**:

- **Binding antibody**: Goat anti-human IgG (1 ml, Sigma Aldrich, cat. # I2136-1ML). Lot 017M4800V: 2.2 mg/ml, diluted to 1 mg/ml in dilution buffer [added 1.2 ml] and stored in 50 µl aliquots at -20°C (1:1000). Discard what is not used of the aliquot.
- **Detection antibody**: HRP-conjugated donkey anti-human IgG (500 µl, BioLegend, cat # poly24109; undiluted Ab [0.32 mg/ml] is stored at 4°C and should be used at 1:1000). Return antibody to fridge after use.
- **Protein for standard curve**: Human IgG (10 mg, Sigma Aldrich, cat. # I2511-10MG). Lot 046M4855V: 5.5 mg/ml, diluted to 1 mg/ml in dilution buffer [added 8.18 ml] and stored in 100 µl aliquots at -20°C. **Write the date of thawing on the side of the tube and store at 4°C (Antibody Box 1, position I9). Do not use for more than 1 month after thawing**.

Procedure		**Incubation**
Step 1:	Coating	1 hour OR O/N
Step 2:	Blocking	1 hour OR O/N
Step 3:	Antigen Binding	2 hour
Step 4:	Detection antibody	1 hour
Step 5:	Visualization	0.5 hour
Step 6:	Stopping	
Step 7:	Measure	
Step 8:	Disposal	

Step 1: Coating (volumes for all steps are for 1 plate)

- Prepare ~10.5 ml coating buffer:
  - 10.5 ml PBS
  - 42 µl of 1 mg/ml coating antibody (4 µg / ml final concentration)
- Add 100 µl coating buffer to each well
- Cover the plate with the lid (also during subsequent incubation steps)
- Incubate **1 hour at 37°C** or **O/N at 4°C**

Step 2: Blocking

- Prepare 16 ml of blocking buffer:
  - 5.3 ml NAP buffer + 10.7 ml PBS
- Wash the coated plate once with **deionized** water using the special tap in the sink. After the wash, forcefully hit the plate on a stack of paper towels to make sure the wells are completely dry.
- Add 150 µl of blocking buffer to the coated wells
- Incubate the plate for **1 hour at 37°C** or **O/N at 4°C**

Note. Coated or blocked plates can be stored under wet conditions (meaning wells containing coating or blocking solution) at 4°C for several weeks. Each plate needs to be sealed with a plastic 96 wells plate sticker.

Step 3: Antigen-binding

- Wash all wells **3** times with **deionized** water. After each wash, forcefully hit the plate on a stack of paper towels to make sure the wells are completely dry.
- Prepare ~15 ml dilution buffer:
  - 15 ml PBS
  - 150 µl NAP buffer
- Transfer 50 µl dilution buffer to each well
- Transfer 50 µl sample or standard (see below) to each well. (Note: for certain applications, it may be required to add 20 µl of sample to 80 µl of dilution buffer).
- Transfer 50 µl dilution buffer to wells that do not contain sample nor standard
- Incubate for **2 hours at 37°C**.

Making standard curve (two [= duplicate] on each plate when high accuracy is required, otherwise duplicate or triplicate on one of the plates [when you have multiple plates])

- Prepare duplicate three-fold serial dilutions (ten) of human IgG protein standard in a range between 20 µg/ml and 1 ng/ml (20,000 / 6,667 / 2,222 / 741 / 247 / 82 / 27 / 9 / 3 / 1 ng/ml)
- Start with diluting the aliquot (1 mg/ml) to 20 µg/ml (hence 50 x dilution) using dilution buffer (1% NAP buffer in PBS) in a 1.5 ml tube (e.g. 5 µl 1 mg/ml IgG in 245 µl dilution buffer)
- Also use dilution buffer for subsequent dilution steps; the 15 ml will be enough also for the dilutions of the standard). Make 3-fold dilutions (e.g. 55 µl + 110 µl dilution buffer) using 10% more than required in the wells (e.g. 110 µl for 2 wells).
- Use new tips for each new dilution!

Step 4: Detection antibody

- Wash the plate **5** times with **deionized** water. After each wash, forcefully hit the plate on a stack of paper towels to make sure the wells are completely dry.
- Prepare the secondary antibody solution:
  - 10 ml of PBS
  - 100 µl of NAP buffer

    - 10 µl of 0.32 mg/ml HRP-conjugated anti-human IgG (stored at 4°C, return to fridge a.s.a.p.) (BioLegend 410902; Antibody Box 1, B2)
- Add 100 µl of detection antibody solution to each well
- Incubate the plate for **1 hour at 37°C**.

Step 5: Visualization

- Wash (see step 3)
- Add 100 µl TMB substrate solution per well and incubate in the dark at RT until the blue color reaction of standard series are well detectable (5 – 30 min)

Step 6: Stopping

- Stop the reaction by adding 100 µl 0.18 M H_2SO_4 per well
- Cover each plate with a transparent plastic 96 well plate sticker. Make sure to remove the overhangs of the sticker.

Step 7: Measure

- Measure the absorbance at 450 nm for 0.1 sec using the ELISA reader in the Leadbetter lab

Step 8: Disposal

- Temporarily store the plates next to Sebastiaan's desk. He will contact EH&S for proper disposal of the chemical waste.

General informative video about ELISAs:

https://www.youtube.com/watch?v=YJ0-qQslqqQ