Polyreactivity ELISA

Materials

Antigens

Aliquots stored at -20°C:
dsDNA from calf thymus (Sigma, D8515)
ssDNA prepared from dsDNA (heat at 95°C for 30 min)
LPS from *E. coli* (Sigma, L2637)
KLH (Sigma, H8283)
Cardiolipin (Sigma, C0563)

Aliquots stored at 4°C:
Human insulin (Sigma, I9278)

Reagents & Consumables

HRP-conjugated goat anti-human IgG (Jackson, 109-035-098)
ABTS solution (Invitrogen, 00-2024)
96-well High-binding EIA/RIA plate, flat bottom, polystyrene (Costar, 9018)
Polypropylene 1.2 ml microtiter 96-well-format tubes (USA Scientific, 1412-1000)
1X PBS
200-proof pure ethanol
Filtered water ($H_20^{(f)}$)

ELISA Buffer

0.5M EDTA, pH 8	4 ml
10X PBS	200 ml
Tween	1 ml
$H_20^{(f)}$	1800 ml

Method

DAY 1
1. Antigen coating

- Prepare ssDNA by heating dsDNA at 95°C for 30 min
- Dilute stock solutions of dsDNA, ssDNA, LPS & KLH (1 mg/ml) at 1:100 in 1X PBS (5 ml for 1 plate)
- Dilute stock solution of Insulin (10 mg/ml) at 1:2000 in PBS (5 ml for 1 plate)
- Dilute stock solution of CL (1 mg/ml) at 1:100 in pure ethanol (5 ml for 1 plate)

- Add 50 µl/well (final concentration of 10 µg/ml for dsDNA, ssDNA, LPS, KLH and CL, and 5 µg/ml for Insulin)
- Incubate overnight at room temperature (RT)
    - Cover plates with parafilm, except CL: needs to dry out overnight, so do not cover

DAY 2
- Wash plates 3x with filtered water ($H_20^{(f)}$) – 200µl/well if using a multichannel, or fill the well if using the Immunowasher
    -Discard previous reagents from plates before wash steps

2. ELISA buffer incubation

- Add 200 µl of ELISA buffer/well

- Incubate for 1 to 2 h (RT); in the meantime, prepare samples & dilutions [step 3]

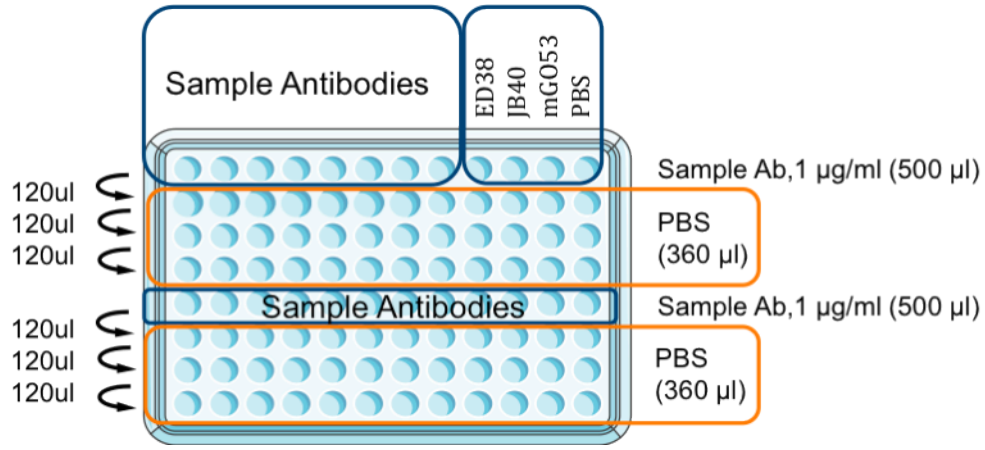
- When ELISA buffer incubation is done and sample dilutions are ready, wash 3x with $H_20^{(f)}$

3. Sample antibody incubation

- Test IgG samples/controls at 1 µg/ml and 3 consecutive 1:4 dilutions in PBS; make at least 350 µl of each sample dilution

    - First make 100 µl of a 100 µg/ml stock in PBS, in microcentrifuge tubes
    - Prepare rest of samples in 96-well tubes/deep block format
        - Make 500 µl of 1µg/ml [5 µl 100 µg/ml stock + 495 µl PBS]; this is the first sample
        - Then make 3 1:4 serial dilutions with 120 µl of previous sample dilution + 360 µl PBS; these are samples 2, 3 and 4

Sample Prep Diagram:



- Add 50 µl of each sample to each antigen plate; incubate for 2 h (RT)

- Wash 3x with $H_20^{(f)}$

4. HRP-conjugated secondary antibody incubation

- Prepare HRP-conjugated goat anti-human IgG antibody (stock 0.8 mg/ml) at 1:1000 in ELISA buffer (5 ml for 1 plate)

- Add 50 µl/well; incubate for 1 h (RT)

- Wash 3x with $H_20^{(f)}$

5. Substrate Reaction & OD measurement

- Add 200 µl of ELISA buffer/well; incubate for 5 min

- Wash 3x with $H_20^{(f)}$

- Add 100 µl/well of ABTS solution
    - Do one plate at a time: add substrate to 1 plate and complete all OD readings, then add substrate to the next plate and read

- Make several successive readings at 405nm

- Subtract OD^{SAMPLE} by OD^{PBS}

    High positive control ed38 OD 405nm = 3 to 3.5
    Low positive control eiJB40 OD 405nm = 0.5 to 1.5
    Negative control mgo53 OD 405nm = below 0.5