

## ELISA Protocol for Lewis antigen determination in Hp

Reagents:

**10XPBS:**

| Reagent                          | In 1L | In 500mL |
|----------------------------------|-------|----------|
| NaCl                             | 80g   | 40g      |
| KH <sub>2</sub> PO <sub>4</sub>  | 2g    | 1g       |
| Na <sub>2</sub> HPO <sub>4</sub> | 6.1g  | 3.05g    |
| KCl                              | 2g    | 1g       |
| ddH <sub>2</sub> O               | To 1L | To 500mL |

Autoclave 30m/15psi/121°C, store at room Temp.

**CC Buffer (0.05M Sodium Bicarbonate):**

| Reagent                         | In 1L    | In 500mL |
|---------------------------------|----------|----------|
| Na <sub>2</sub> CO <sub>3</sub> | 1.59g    | 0.795g   |
| NaHCO <sub>3</sub>              | 2.93g    | 1.465g   |
| NaN <sub>3</sub>                | 0.20g    | 0.10g    |
| ddH <sub>2</sub> O              | To 900mL | To 450mL |

Adjust pH to 9.6, take to final volume, filter sterilize, store @ 4°C

**1XPBS-AT (PBS-Azide/Tween):**

| Reagent            | In 1L | In 2L |
|--------------------|-------|-------|
| 10XPBS             | 100mL | 200mL |
| NaN <sub>3</sub>   | 0.20g | 0.40g |
| Tween20            | 0.5mL | 1mL   |
| ddH <sub>2</sub> O | To 1L | To 2L |

Store @4°C, no need to be sterile

**1XPBS-A-2%BSA Blocking solution (Azide-Bovine serum albumin)**

| Reagent            | In 100mL | In 500mL |
|--------------------|----------|----------|
| 10XPBS             | 10mL     | 50mL     |
| NaN <sub>3</sub>   | 0.02g    | 0.1g     |
| BSA                | 2g       | 10g      |
| ddH <sub>2</sub> O | To 100mL | To 500mL |

**1XPBS-A-1%BSA Blocking solution (Azide-Bovine serum albumin)**

| Reagent            | In 100mL | In 500mL |
|--------------------|----------|----------|
| 10XPBS             | 10mL     | 50mL     |
| NaN <sub>3</sub>   | 0.02g    | 0.1g     |
| BSA                | 1g       | 5g       |
| ddH <sub>2</sub> O | To 100mL | To 500mL |

Resuspend both completely, filter sterilize, store @ 4°C

**Solution for peroxidase substrate (tablets) (0.1M diEtOhamine-HCL buffer):**

| Reagent            | In 1L    | In 100mL |
|--------------------|----------|----------|
| Diethanolamine     | 97mL     | 9.7mL    |
| ddH <sub>2</sub> O | To 800mL | To 80mL  |

Adjust pH to 9.8 using 1M HCl, bring the final volume, filter sterilize, store @ 4°C. To use add 1 tablet of pNPP to 5mL of solution **1h** before start ELISA

**Saline-15%Glycerol:**

| Reagent                   | In 50mL | In 100mL |
|---------------------------|---------|----------|
| Sterile saline (0.9 NaCl) | 42.5mL  | 85mL     |
| Sterile glycerol          | 7.5mL   | 15mL     |

Vortex until all glycerol is re-suspended, maintain sterile.

**A. Collect cells**

1. Grow 1-2 plates of each strain to be tested in TSA-10%SB plates at 37°C for 48h.
2. Harvest the plates in 1ml of sterile solution
3. Spin @ 8krpm for 5m.
4. Discard supernatant, resuspend in 1mL of sterile saline
5. Split into two tubes, label one for ELISA (dark blue borders) and one for DNA prep (green sides)
6. Spin @8krpm for 5 min.
7. Discard supernatants, resuspend the ELISA aliquot in 100µL of Saline-15%glycerol solution and the DNA prep one in 560µL TE and 30µL 10%SDS, mix well.
8. Can be stored @-20°C.

**B. Protein determination (using BCA Pierce kit):**

1. Prepare 1 glass tube for each sample, 5 glass tubes for standards, and 1 tube for blank
2. Prepare standards:
  - i. Label tubes 1→5. Add:
    1. 100µL of BSA 2mg/ml
    2. 100µL of BSA 2mg/ml + 100µL sterile dH<sub>2</sub>O
    3. 100µL from tube 2 + 100µL sterile dH<sub>2</sub>O
    4. 100µL from tube 3 + 100µL sterile dH<sub>2</sub>O
    5. 100µL from tube 4 + 100µL sterile dH<sub>2</sub>O (then discard 100 µL of the solution)
3. Prepare samples:
  - i. Mix in the tubes 90µL of sterile dH<sub>2</sub>O and 10µL of sample
4. Mix BCA protein assay reagents A and B 50:1 respectively, enough for all your samples, standards, blank +~5. Add 2mL

of the BCA protein assay reagents mix to each tube. (1960µL of A and 40µL of B per sample)

5. Incubate **30m** @ 37°C
6. Read in the spectrophotometer:
  - i. Click on Protein → Vis-ON → Work → Choose HPW
  - ii. Put blank in and click on "Read blank"
  - iii. Read standards in this order: 5,4,3,2,1. Click on "Read 1"
  - iv. To see standard curve, click on "display standard curve"; print.
  - v. Load samples on the machine. Click on "Samples" and start to read samples
  - vi. Make sure the Auto sampler option is in 6
  - vii. Leave "1" as dilution factor
  - viii. Print
7. For each sample, calculate the protein concentration:

$$[1000/(\text{Conc} \mu\text{g} \times 100)] \times 10 = X$$

$$X - 1000 = Y$$

Where  $X$  = volume of my sample to use;  
 $Y$  = Volume of CC buffer to dilute  $X$  in

### C. Lewis determination ELISA

1. Label 2 glass tubes for each samples with the name of the sample and 1 or 2.
  - i. Tube 1: add 990 µL of CC Buffer + 10 µL of sample.
  - ii. Tube 2:  $X$  volume from Tube 1 +  $Y$  volume of CC buffer.
2. Label the plates (Le<sup>A</sup>, Le<sup>B</sup>, Le<sup>X</sup>, Le<sup>Y</sup>).
3. In the first two wells (A1 and A2) add negative Le control (HB101 cells).
4. Add 100 µL of each sample to each well (2 wells for each sample) in columns.
5. Allow the plate to incubate overnight @ room temperature.
6. Discard coating solution.
7. Add 200µL of 1XPBS-A-2%BSA blocking solution to each well. Shake and incubate for over **1h**.
8. Discard blocking solution. **Don't wash.**
9. Make dilutions of the primary antibodies in 1XPBS-A-1%BSA solution:
  - i. Anti- Le<sup>X</sup>: 1:500 (20µL/10mL per plate)
  - ii. Anti- Le<sup>Y</sup>: 1:1000 (10µL/10mL per plate)
  - iii. Anti- Le<sup>A</sup>: 1:500 (20µL/10mL per plate)

- iv. Anti- Le<sup>B</sup>: 1:1000 (10µL/10mL per plate)  
Add 100µL to each well. Check that all wells have the Ab.  
Shake for **1h** @ room temp.
10. Discard the primary antibody solution. Use the Automated Strip Washer machine. Remember to prime if a different buffer has been used in the machine.
- i. Prime the machine with buffer PBS-AT - Place input tube on PBS-AT bottle, press **Main Menu** button, then **Run**, **Prime**, choose program **08 BAH**, press **Enter** then **Start**. Repeat once more.
  - ii. Wash the Plates – After priming, put the plate in the plate holder, with A1 facing the back right corner. Close the lid and press **Run**, **Wash**, choose program **06 CABE**, and choose the **Number of Stripes** (i.e. the number of columns). Press **Enter** then **Start**.
11. Make dilution of the secondary Ab solution (dilute in PBS-AT):
- i. Le<sup>X</sup>: anti-mouse IgM 1:2500 (4µL/10mL per plate).
  - ii. Le<sup>Y</sup>: anti-mouse IgM 1:2500 (4µL/10mL per plate).
  - iii. Le<sup>A</sup>: anti-mouse IgG 1:5000 (8µL/10mL per plate).
  - iv. Le<sup>B</sup>: anti-mouse IgM 1:2500 (4µL/10mL per plate).
- Add 100 µL to each well, shake for **1h**.
12. Discard and wash using the Automated Strip Washer machine (see 10).
13. Add substrate:
- i. Dissolve 2 tablets of pNPP in 10mL of 0.1M diEtOhamine-HCL buffer. Add 100µL in each well. Incubate in a dark place, at room temp. for **1h**.
14. Read ELISA
- i. Click on "**Revelation**".
  - ii. Put plate in correct position, with A1 in the top left corner.
  - iii. Click on **File**, **Read Plate**, **ED\_LEWIS**, **OK**, name the plate on the screen and press **OK**, **File**, **Print**.