Thursday, March 19, 1998

Protocol for Histamine-Elisa Kit from IBL

Before starting check if:

1- Is the **assay buffer** prepared? Dilute the assay buffer concentrate 1:5 with dd H_2O. Store at 2-8°C

2- Is the **wash buffer** prepared? Dilute the wash buffer concentrate 1:20 with dd H_2O.

3- Is the **enzyme conjugate** ready? 5µl of enzyme conjugate concentrate in 1.0 ml of assay buffer. Must be made fresh every day.

I- ACYLATE STANDARDS, CONTROLS, AND SPECIMENS

1- Get one 3 ml glass tube per sample and identify

2- Fill glass tubes with:

50 µl of U/Z **standards** (A to F) or **control urine** or **rat urine** in appropriate tube

50 µl of **indicator buffer** in each tube (must stay pink otherwise adjust pH)

10 µl of **acetylation reagent** in each tube

3- Incubate a room temp for **30 min**.

4- Add 2 ml of **assay buffer** (*diluted*) and vortex mix.

II- IMMUNOREACTION

1- Pipet **50 µl** of acetylated standards, control, and rat urine in appropriate well

2- Add **50 µl** of **enzyme conjugate** (***use diluted conjugate!***)

3- Add **50 µl** of **antiserum** (green)

4- Seal and incubate on shaker for **3 hours**

III- WASHING

Wash each well 4 times with 250 µl of **wash buffer** (*diluted*)

IV- TMB reaction

1- Prepare TMB substrate solution: 100µl of **TMB substrate concentrate** + 3 ml of **TMB Substrate buffer** and mix.

2- Pipet **200µl per well**

3- Seal and incubate for **20 min** (urine) on shaker

4- Stop by adding 100µl of stop solution into each well.

V- Read density at 450 nm