Ghrelin concentration human serum

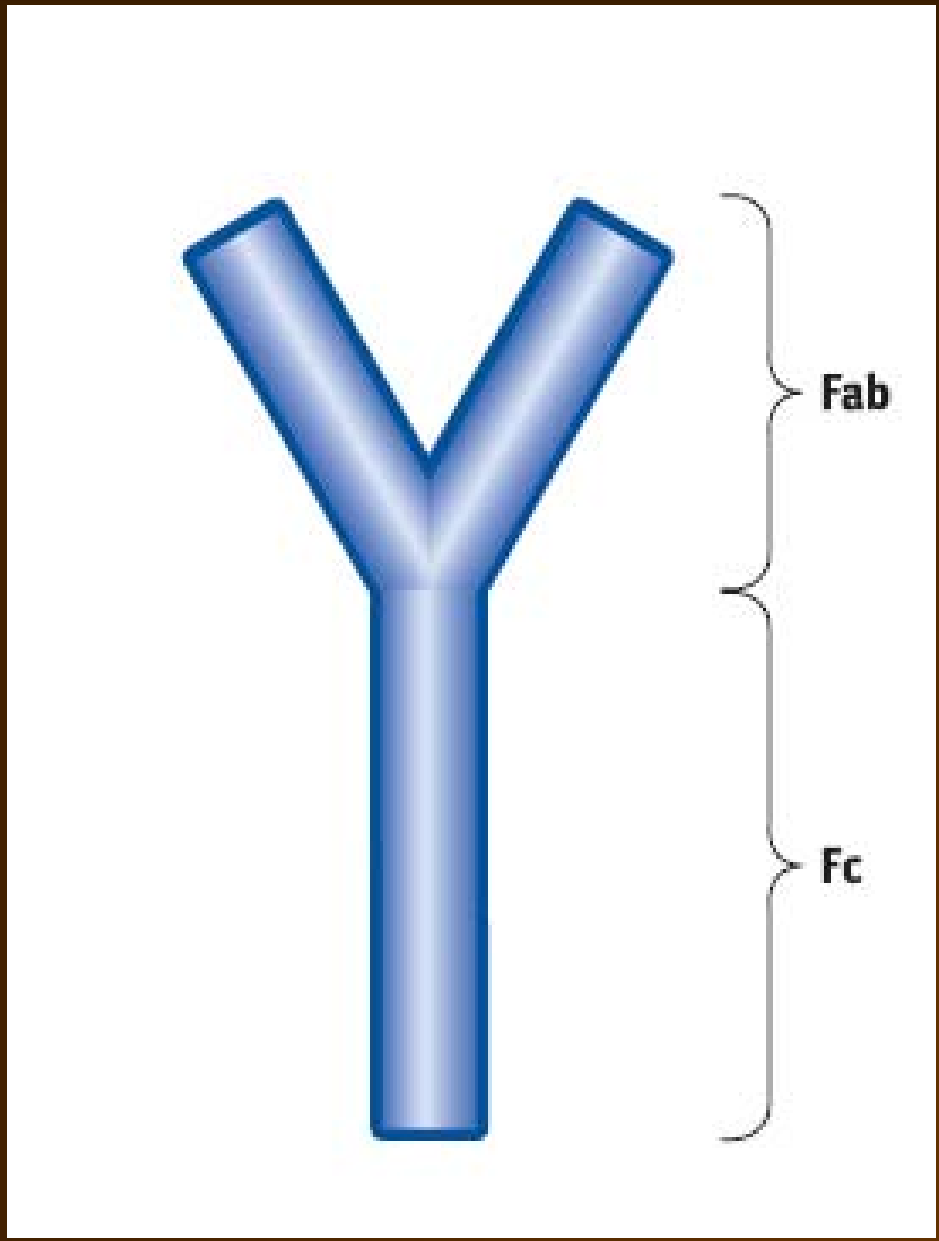
GENERAL PROTOCOL FOR ENZYME IMMUNOASSAY KIT

STORAGE:

- Store the kit at 2 - 4°C upon receipt.
- The kit will be stable for 6 months.
- The kit should be equilibrated to room temperature before assay.
- It is recommended that the solutions be used on the same day of rehydration

GENERAL INFORMATION

- :
- The immunoplate in this kit is pre-coated with secondary antibody and the non specific binding sites are blocked.
- The secondary anti body can bind to the ***Fc*** fragment of the primary antibody (peptide antibody) whose ***Fab*** fragment will be competitively bound by both biotinylated peptide and peptide standard or targeted peptide in samples.


Fab
Fc

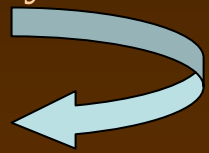
- The biotinylated peptide is able to interact with streptavidin horseradish peroxidase (SA-HRP) which catalyzes the substrate solution composed of 3,3’.5,5’-tetramethylbenzidine (TMB) and hydrogen peroxide to produce a blue colour solution.

- The enzyme- substrate reaction is stopped by hydrogen chloride (HCI) and the solution turns to yellow. The intensity of the yellow is directly proportional to the amount of biotinylated peptide-SA-HRP complex but inversely proportional to the amount of the peptide in standard solutions or samples.

- This is due to the competitive binding of the biotinylated peptide and the peptide in standard solutions or samples to the peptide antibody (primary antibody). A standard curve of a peptide with known concentration can be established accordingly. The peptide with unknown concentration in samples can be determined by extrapolation to this standard curve.

- Peptide
- (in standard solutions or samples)

- Immunoplate—Secondary antibody— Primary antibody
- Biotinylated
- Peptide — SA-HRP—Substrate



ASSAY CONDITIONS:

- Plasma, serum, culture media, tissue homogenate, CSF, urine or any biological fluid can be assayed as long as the level of the sample is high enough for the sensitivity of the kit to detect it.

GENERAL PROCEDURE FOR UTILIZATION OF THE EIA KIT:

- 1 Thoroughly read this protocol before performing an assay.
- 2. Dilute the assay buffer concentrate with 950m1 of distilled water.
- This assay buffer will be used to reconstitute all of the other compounds in this kit and the extract of plasma samples.
- 3. Rehydrate standard peptide with 1 ml assay buffer, vortex.
- The concentration of this stock solution is 1,000ngIml.

4 Prepare peptide standard solutions as follows:

Standard No.	Std. volume	Assay Buffer	Concentrations
Stock	1.000µl	----------	1000ng/ml
#1	100 µl Stock	900	100ng/ml
#2	100 µl Stock #1	900	10ng/ml
#3	100 µl Stock #2	900	1ng/ml
#4	100 µl Stock #3	900	0.1ng/ml
#5	100 µl Stock #4	900	0.01ng/ml

- 5. Rehydrate primary antiserum with 5m1 of assay buffer, vortex.
- 6. Rehydrate biotinylated peptide with 5m1 of assay buffer, vortex.
- 7. Leave well A-l empty as **Blank.**

- 8. Add 50µl assay buffer into well B- 1 as Total Binding.

- 9. Add 50µl of the prepared peptide standard solutions from #5 to #1 (reverse order of serial dilution) into the wells from C-1 to G-l respectively.

- 10. Add 50µl samples into their designated wells.

- 11. Add 25µl rehydrated primary antiserum into each well except the Blank well.

- 12.Add 25µl rehydrated biotinylated peptide into each well **except** the **Blank** well.
- 13.Seal the immunoplate with acetate plate sealer (APS).
- 14.Incubate the immunoplate for 2 hours at room temperature.

- 15. Centrifuge the SA-HRP vial provided in this kit (500-1,000 r.p.m.. 15 seconds, 4°C) and pipet l2µl SA-HRP into 12ml assay buffer to make SA-HRP solution. vortex.

- 16. Remove APS from the immunoplate.

- 17. Discard contents of wells.

- 18. Wash each well (except the **Blank)** with 300µl assay buffer, discard the buffer and blot dry the plate. Repeat 5 times.

- 19.Add 100µl SA-HRP solution into each well except the **Blank** well.
- 20.Reseal the immunoplate with APS. Incubate for 1 hour at room temperature.
- 22.Wash and blot dry the immunoplare 6 times with the assay buffer as described above.
- 23.Add l00µl substrate solution provided in this kit into each well including the **Blank** well.
- 24.Reseal the immunoplate with APS.

- 25. Incubate for 1 hour at room temperature.
- 26. Add 100µl 2N HCI into each well (including the **Blank**) to stop the reaction. Go to the next step within 20 minutes.

- 27. Clean the immunoplate bottom with 70% ethanol.

- 28. Remove APS and load the immunoplate onto a Microtiter Plate Reader.

- 29. Read absorbance O.D. at 450nm.

SUMMARY OF ASSAY PROTOCOL

Add 50µl well of standard or sample, 25µl primary antiserum and 25µl biotinylated peptide.

▼

Incubate at room temperature for 2 hours

▼

Wash immunoplate 5 times with 300µl/well of assay buffer

▼

Add l00µl /well of SA-HRP solution

▼

Incubate at room temperature for 1 hour

▼

Wash immunoplate 6 times with 300µl /well of assay buffer

▼

Add l00µl of substrate solution

▼

Incubate at room temperature for 1 hour

▼

Terminate reaction with 10011L'well of 2N HCl

▼

Read absorbance O.D. at 450nM and calculate results

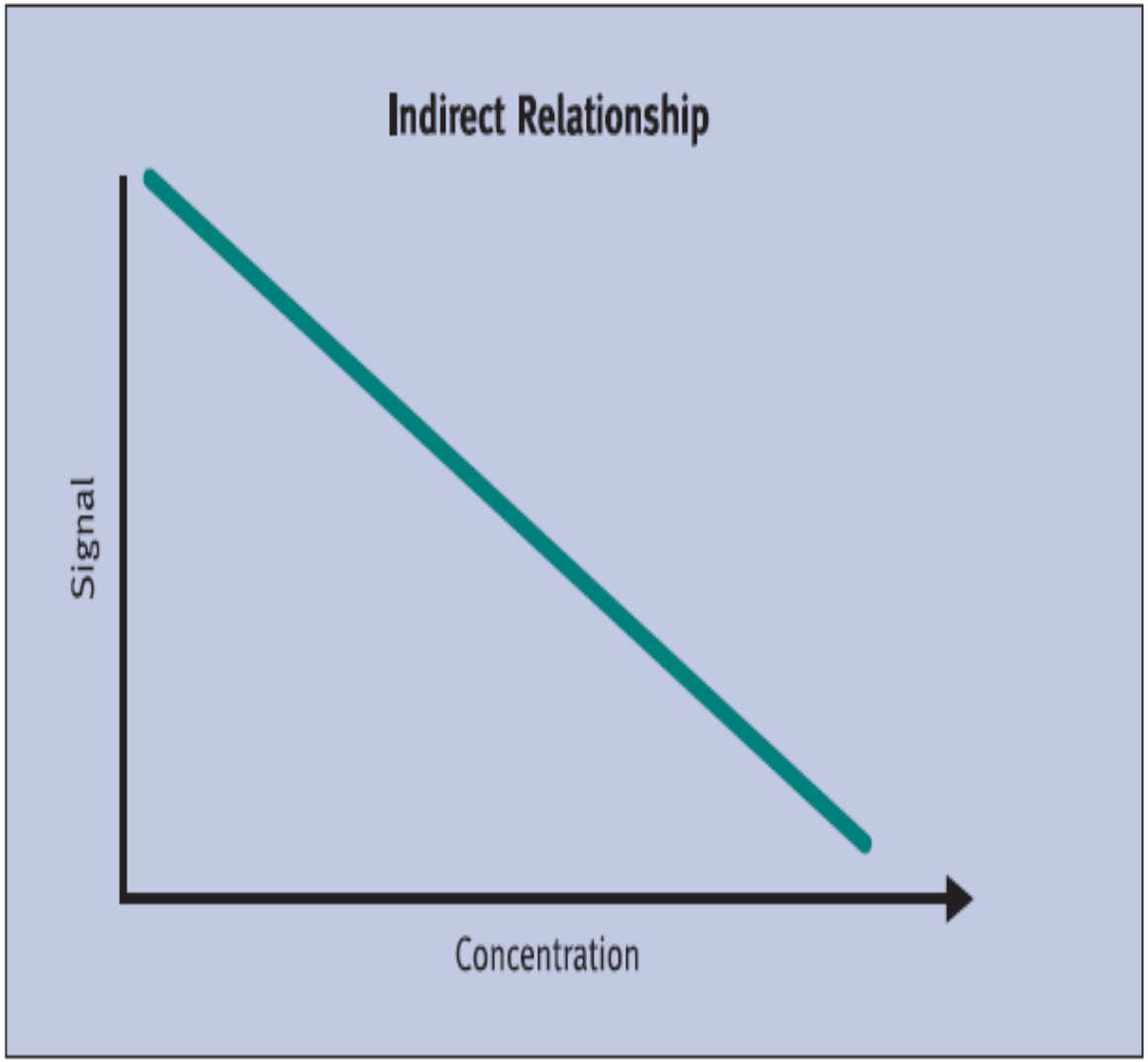


FIGURE 1-7
Amount of antigen is indirectly related to the amount of label (signal) in competitive formats

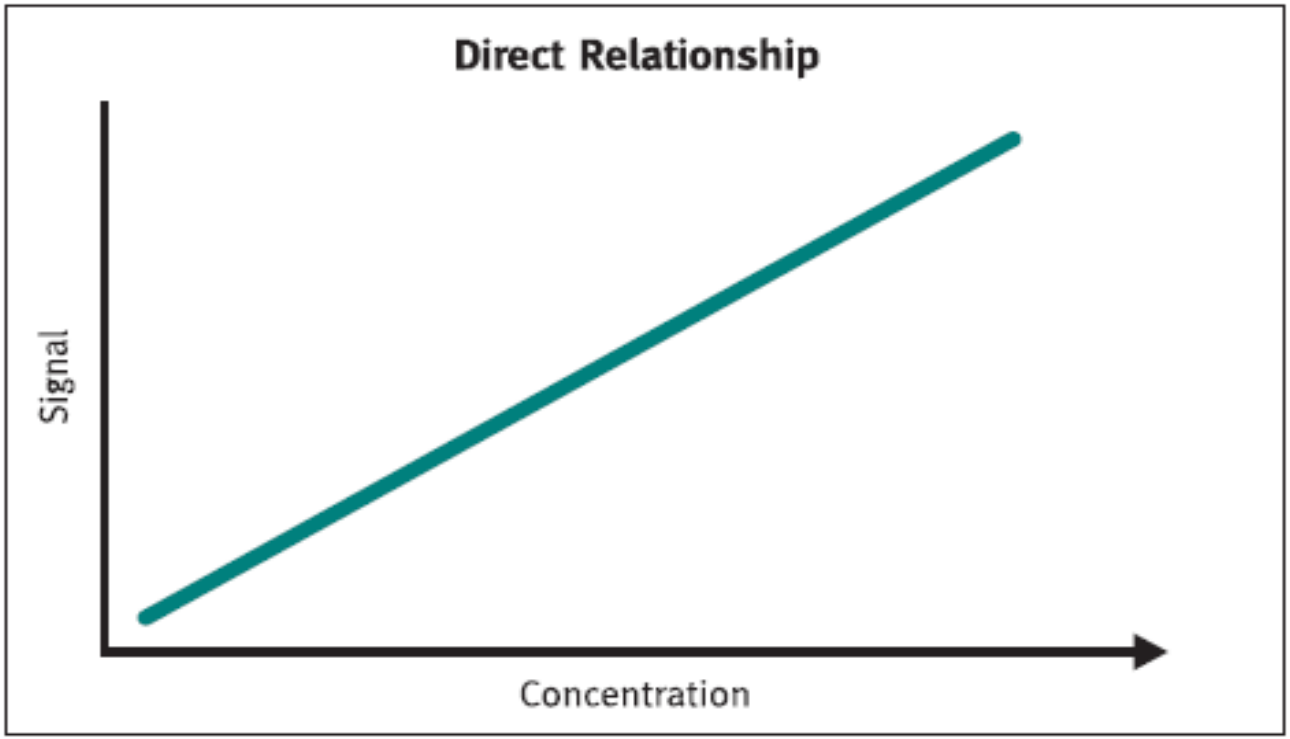


FIGURE 1-11 Amount of antigen is directly related to the amount of label (signal) in competitive formats

Any question

Thank you