ECL #K-1200

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For Investigational Use Only

Hyaluronan Enzyme-Linked Immunosorbent Assay Kit (HA-ELISA)

Product No: K-1200

INTENDED USE: THIS PRODUCT IS FOR RESEARCH USE ONLY. NOT INTENDED FOR CLINICAL OR DIAGNOSTIC USE.

Kit includes:

Incubation plate
HA ELISA Plate
HA Standard (3.2 µg/ml)
Detector
Substrate Buffer
Substrate Pellet
Stop Solution
Wash Concentrate 10X
Enzyme
Diluent

Researcher must provide:

Absorbance microplate reader
37°C Incubator
Pipettes
Plate Cover

Storage and Stability

Kit can be stored unopened at 4°C for up to six months. Opened and reconstituted solutions, except the working substrate solution, can be used for up to two months when stored at 4˚C. The working substrate solution should be aliquoted and stored at -20°C. All components and solutions should be protected from light.

Background

Hyaluronan (HA) is a high molecular weight (1000-5000 kD) anionic polysaccharide composed of repeating disaccharides of glucuronate acetylglucosamine. The HA-ELISA is a quantitative enzyme-linked immunoassay designed for the *in vitro* measurement of HA levels in human or animal biological fluids (blood, serum, urine, diffusate, synovial fluid) or cell-culture supernatant.

The HA-ELISA is a competitive ELISA assay in which the colorimetric signal is inversely proportional to the amount of HA present in the sample. Samples to be assayed are first mixed with the Detector, then added to the HA ELISA Plate for competitive binding. An enzyme-linked antibody and colorimetric detection is used to detect the HA detector bound to the plate. The concentration of HA in the sample is determined using a standard curve of known amounts of HA.

Reagent Preparation

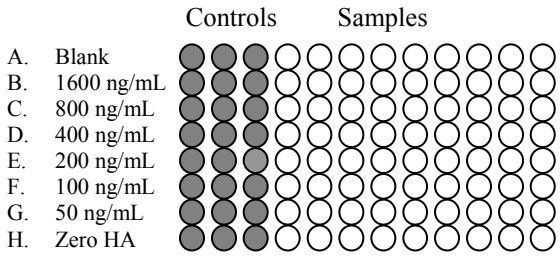
HA Standards: Make 1:2 serial dilutions of the HA Standard using the Diluent to obtain standards of 1600, 800, 400, 200, 100, and 50 ng/mL (Controls may be diluted in the plate, following the diagram below).

Working Detector: Dilute Detector with 5 mL Diluent.

Working Enzyme: Dilute Enzyme with 10 mL Diluent.

Wash Buffer: Make a 1:10 dilution of Wash Buffer in distilled water.

Working Substrate Solution: Dissolve Substrate Pellet in 10.5 mL Substrate Buffer.



Incubation Plate

Assay Procedure

1. Set up the incubation plate (blue U-bottom plate) as illustrated above. We suggest the HA Standard dilution series be run in triplicate for best results. Add 100 µL of Standards and samples into corresponding wells. Add 150 µL of Diluent to Blank Control and 100 µL of Diluent to Zero HA Control wells. Add 50 µL of Working Detector to all wells except the Blank. Mix well. Cover plate and incubate for one hour at 37°C.

2. Following the incubation, transfer 100 µL of controls and samples to the corresponding wells of the HA ELISA plate. (This is easily accomplished with a multi-channel pipettor.) Cover plate and incubate for 30 minutes at 4°C.

3. Discard the solution and wash the wells four times with 300 µL of 1X Wash Buffer.

4. Add 100 µL of Working Enzyme to each well. Cover plate and incubate at 37°C for 30 minutes.

5. Repeat wash step 3.

6. Add 100 µL Working Substrate Solution to each well. Incubate the plate in the dark at room temperature for 30-45 minutes

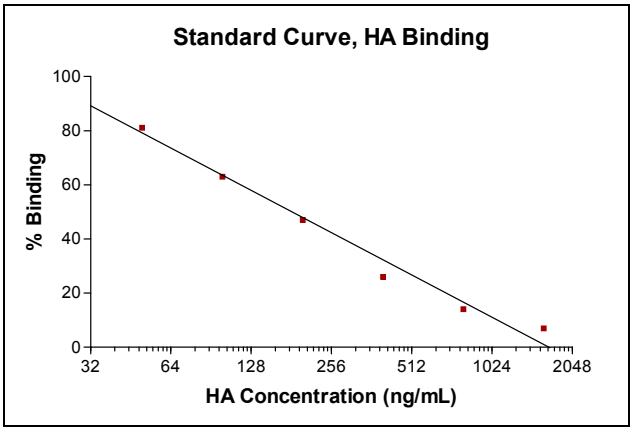
7. Measure the absorbance of each well at 405 nm. The Blank should have an absorbance of $\leq$ 0.10 and the ratio of the Zero HA Control to the 1600 ng/mL HA Standard should be >4.0. If the ratio is<4.0, continue incubation and read plate every 15 minutes until ratio is reached.

8. Stop the reaction by adding 50µL Stop Solution to each well.

9. Calculate the binding percentage for each sample using the formula:

$$[A_{405}(\text{Sample}) - A_{405}(\text{Blank})] / [A_{405}(\text{Zero HA}) - A_{405}(\text{Blank})] \times 100 = \%\ \text{Binding}$$

Using linear or nonlinear regression, plot a standard curve of percent binding versus concentrations of HA standards. A Log2 plot with linear regression is shown as an example. Determine HA levels of unknowns by comparing their percentage of binding relative to the standard curve.



Research Reference Values

Normal HA levels in serum from healthy blood donors are less than 120 ng/mL. Serum HA levels are elevated in several disease states including hepatitis (greater than 160ng/mL) and cirrhosis (greater than 250ng/mL).

References

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