Indole 3 Acetic Acid (IAA) CLIA Kit

Catalog No: abx190011

Size: 96T

Range: 1.56 ng/ml - 400 ng/ml

Sensitivity: < 0.59 ng/ml

Storage: Store the 96-well plate, Standards, and Detection Reagent(s) at -20°C, and the rest of the kit components at 4°C.

Application: The quantitative detection of IAA in Tissue and cell culture supernatant.

Principle of the Assay: This kit is based on competitive chemiluminescent immunoassay technology. An antibody is pre-coated onto a 96-well plate. Standards, test samples, and biotin-conjugated reagent are added to the wells and incubated. A competitive inhibition reaction takes place between the biotin-labelled IAA and the unlabelled-IAA on the pre-coated antibody. The HRP-conjugated reagent is then added, and the whole plate is incubated. Unbound conjugates are removed using wash buffer at each stage. After the Substrate working solution is added, only wells that contain the labelled IAA will produce chemiluminescence. The intensity of the emitted light is inversely proportional to the amount of IAA in the sample.

Kit Components
- Pre-coated 96-Well Microplate: 12 x 8
- Standard: 2 tubes
- Wash Buffer: (30X) 20 ml
- Standard Diluent Buffer: 20 ml
- Detection Reagent A: (100X) 120 µl
- Detection Reagent B: (100X) 120 µl
- Diluent A: 12 ml
- Diluent B: 12 ml
- Substrate A: 10 ml
- Substrate B: 2 ml
- Plate Sealer: 3

Materials Required But Not Provided
- 37°C incubator
- Multi and single channel pipettes and sterile pipette tips
- Squirt bottle or automated microplate washer
- 1.5 ml tubes
- Distilled water
- Absorbent filter papers
- 100 ml and 1 liter graduated cylinders
- 0.01 mol/L PBS (pH 7.0 - 7.2)
- Luminometer capable of reading 96-well microplates (lag time 30.0 secs and read time 1.0 sec/well)

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Protocol

A. Sample Preparation

Analyse immediately or store samples at 2-8°C (within 24 hrs). For long term storage, aliquot and store at -20°C or -80°C. Avoid multiple freeze-thaw cycles.

- **Tissue:** Homogenise 1 g of tissue with 6 ml of 80% methanol solution at 0-2°C for 5 min. Stir the homogenate for 24 hours at 4°C. Filter the homogenate and collect the supernatant. Add 2 ml of 80% methanol solution to the solid residue, and stir for 1 hour at 4°C. Centrifuge at 2000 × g at 4°C for 10 min. Collect the supernatant and add to the supernatant from step 3. Discard the pellet. Evaporate the combined supernatant on a rotary evaporator to approximately 2 ml. Add 1 ml of petroleum ether to the resulting supernatant and mix thoroughly. Allow the liquid to separate into two separate layers, then discard the upper layer.Evaporate the methanol on a rotary evaporator to obtain the aqueous solution. Collect the solution and assay immediately, or aliquot and store at -20°C or -80°C.
- **Cell Culture Supernatant:** Centrifuge at approximately 1000 × g for 20 mins to remove precipitate.

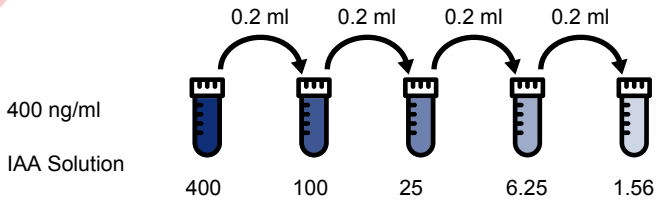
Notes:

- Samples must be diluted so that the expected concentration falls within the kit's range. The sample should be diluted in 0.01 mol/L PBS (PH=7.0-7.2).
- Fresh samples, or recently obtained samples, are recommended to prevent protein degradation and denaturation that may lead to erroneous results.
- NaN_3 cannot be used as a test sample preservative, since it inhibits HRP.
- If possible, prepare solid samples using sonication and/or homogenization, as lysis buffers may (on occasion) interfere with the kit's performance.
- If a sample is not indicated in the manuals applications, a preliminary experiment to determine the suitability of the kit will be required.

B. Reagent Preparation

Standard: Prepare the Standard with 0.5 ml of Standard Diluent buffer to make the 400 ng/ml Standard Solution. This is the highest standard. Allow the reconstituted standard to sit for 10 mins, with gentle agitation prior to carrying out the serial dilutions. Avoid foaming or bubbles. Label tubes in preparation for the serial dilutions - *see the diagram below for reference*. Aliquot 0.6 ml of the Standard Diluent Buffer into each tube (apart from the highest standard tube). Add 0.2 ml of the highest standard solution into the 1st tube and mix thoroughly. Transfer 0.2 ml from the 1st to 2nd tube, mix thoroughly, and so on.

Note: Do not vortex the standard during reconstitution, as this will destabilize the protein. Once the standard has been reconstituted, it should be used within 15 mins. It is not recommended to reuse the reconstituted standard.



Wash Buffer: Dilute the concentrated Wash Buffer 30-fold (1/30) with distilled water (i.e. add 20 ml of concentrated wash buffer into 580 ml of distilled water). If crystals have formed in the concentrated Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.

Detection Reagent A Working Solution Preparation: Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent A 100-fold with Diluent A, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

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Detection Reagent B Working Solution Preparation: Prepare no more than 15 mins before the experiment.

1. Calculate the total volume of working solution required.
2. Dilute Detection Reagent B 100-fold with Diluent B, and mix thoroughly. Pipette with a slow, smooth action to reduce volume errors.

Preparation of Substrate Working Solution:

1. Calculate the total volume of working solution required.
2. In a separate tube, add Substrate A and Substrate B in a 99:1 ratio, respectively (e.g. add 990 µl Substrate A to 10 µl Substrate B). Prepare no more than 10 mins before use.

C. Assay Protocol

Prepare all standards, samples and reagents as directed above. Equilibrate the kit components and samples to room temperature prior to use. It is recommended to measure in duplicate, and to plot a standard curve for each test.

1. Set standard, test sample and control (zero) wells on the pre-coated plate respectively, and record their positions. *Add the solution to the bottom of each well without touching the side walls. Pipette the standards and samples up and down to mix before adding to the wells. Avoid foaming or bubbles.*
2. Aliquot 50 µl of the diluted standards into the standard wells.
3. Aliquot 50 µl of Standard Diluent buffer into the control (zero) well.
4. Aliquot 50 µl of appropriately diluted sample into the test sample wells. Gently tap the plate to mix, or use a microplate shaker.
5. Immediately aliquot 50 µl of Detection Reagent A working solution to each well. Gently tap the plate to mix, or use a microplate shaker. Cover the plate with a plate sealer and incubate for 1 hr at 37°C.
6. Remove the cover and discard the solution. Wash the plate 3 times with 1X Wash Buffer. *Fill each well completely with Wash buffer (350 µl) using a multi-channel Pipette or autowasher (1-2 mins soaking period is recommended). Complete removal of liquid at each step is essential for good performance. After the final wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean absorbent paper towels.*
7. Aliquot 100 µl of Detection Reagent B working solution to each well. Seal the plate and incubate for 30 mins at 37°C.
8. Remove the cover, discard the solution and repeat the wash process as described above, 5 times.
9. Aliquot 100 ul of Substrate working solution into each well. Cover the plate with a plate sealer and incubate for 10 mins at 37°C.
10. Measure the chemiluminescence signal in a microplate luminometer immediately.

This assay is competitive, therefore there is an inverse correlation between the concentration of the sample and the RLUs (Relative Light Units) measured. Create a graph with the log of the standard concentration (Y) and OD measured (X). Apply a best fit trendline through the standard points. Use this graph to calculate sample concentrations based on their OD values. If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Precautions:

- Before using the kit, centrifuge the tubes to bring down the contents trapped in the lid.
- Do not leave the wells uncovered for extended periods between incubations. The addition of reagents for each step should not exceed 10 mins.
- Ensure that the plate is properly sealed or covered during the incubation steps, and that the time and temperature are controlled.
- Do not reuse pipette tips and tubes.
- Do not use expired components, or components from a different kit.
- Please note that this kit is optimised for detection of native samples, rather than recombinant proteins or synthetic chemicals. We are unable to guarantee detection of recombinant proteins, as they may have different sequences or tertiary structures to the native protein.

Precision:

Intra-assay Precision (Precision within an assay): 3 samples with low, medium and high levels of IAA were tested 20 times on one plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, medium and high levels of IAA were tested on 3 different plates, 8 replicates in each plate.

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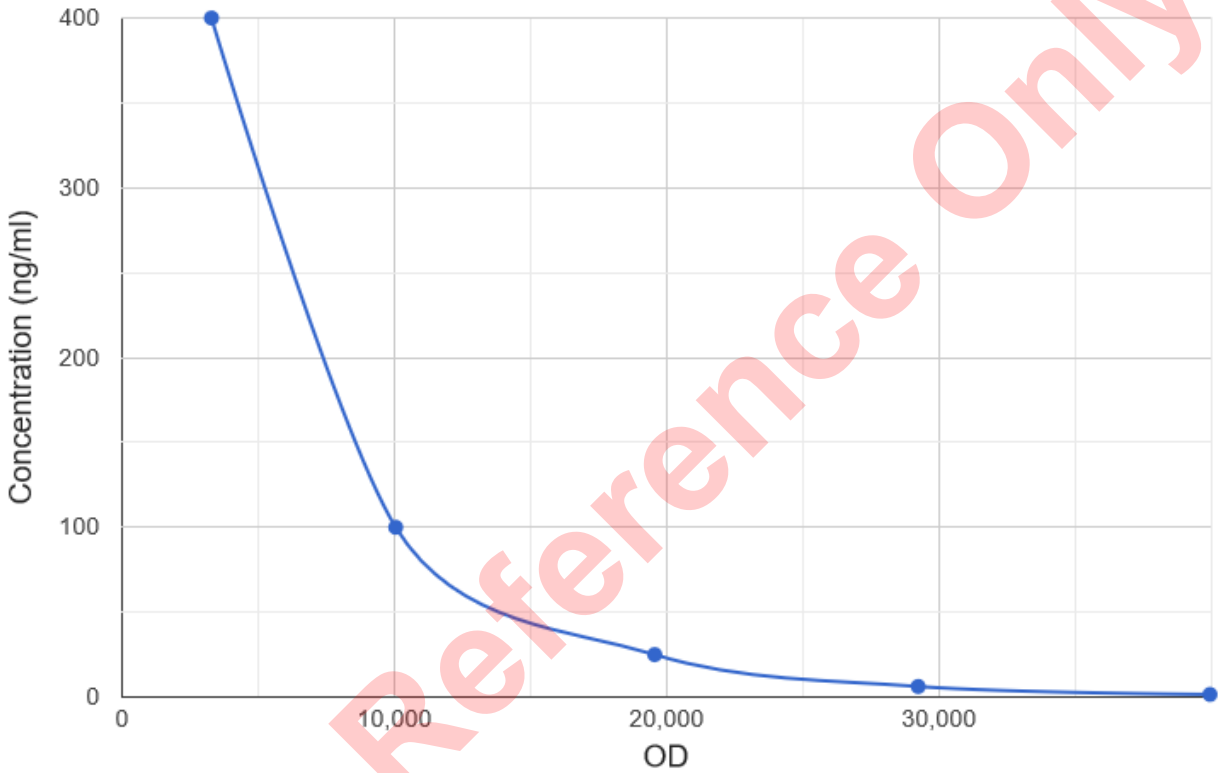
CV (%) = (Standard Deviation / Mean) × 100

Intra-Assay: CV < 10%

Inter-Assay: CV < 12%

D. Typical Data and Standard Curve

Typical Standard Curve Data is provided for demonstration purposes only. A new standard curve must be generated for each assay performed.



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