Phytodetek® IAA Test Kit

Competitive ELISA, for the quantitative determination of Indole-3-acetic Acid
Catalog number: PDK 09346/0096

List of contents

Lot number	Item	96 wells
_____	Hormone standard strips, IAA	3 strips
_____	Anti-IAA coated testwells	96 testwells
_____	IAA-tracer, alkaline phosphatase	0.075 mL
_____	TBS buffer, 20X concentrate	1 bottle – 60 mL
_____	PNP substrate tablets	1 vial – 6 tablets x 5 mg
_____	Substrate diluent (*contains 0.02 % sodium azide*)	1 bottle – 30 mL
_____	PBST wash buffer, 20X concentrate, 50 mL	3 pouches
_____	Instructions	1
	The above items should be stored at 2 - 8 °C.	
	Use within 18 months of receiving	

Materials required, but not provided

- Vertical light path photometer for microtiter plates, strips or wells with 405 nm filter
- Vortex
- 37 °C Incubator - Forced air microplate incubator recommended
- Refrigerator 2 - 8 °C
- 2 - Airtight containers for incubations: One for sample incubation and one for substrate incubation
- Microcentrifuge tubes (ACC 00340) - for preparing standard
- Microcentrifuge tube rack
- Test tubes for standard strip dilution
- Test tube rack
- Distilled water
- Paper towels
- Tweezers or forceps
- Timer
- Scissors
- Additional TBS buffer (ACC 00580) - for sample preparation (see page 7 for buffer formulation)
- Reservoirs - You will need 3 small containers to prepare and hold substrate, wash and tracer solutions
- Pipette tips
- Pipettes
    - Transfer
    - 1 mL volumetric
    - 5 mL serological
    - 100 µL single channel
    - 50-200 µL multichannel

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Principle

Phytodetek enzyme immunoassays are convenient tests for the quantitative determination of plant hormones. Indole-3-acetic Acid (IAA) test utilizes an anti-IAA monoclonal antibody and is sensitive in the range of 78 - 2500 picomoles IAA/mL.

The assay principle uses the competitive antibody binding method to measure concentrations of IAA in plant extracts. IAA-tracer is labeled with alkaline phosphatase and then added along with the plant extract to the antibody coated microwells. A competitive binding reaction is set up between a constant amount of the IAA-tracer, a limited amount of the antibody and the unknown sample containing methylated IAA.

The methylated IAA in the sample competes with the IAA-tracer for antibody binding sites. The unbound IAA-tracer is washed away before adding the substrate. The yellow color produced is inversely proportional to the amount of hormone in the sample. The intensity of color is related to the sample IAA concentration by means of a standard curve.

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Warnings

Phytodetek IAA kit is for research use. Some reagents in the kit contain 0.02 % sodium azide as a preservative. Consult manual guide “Safety Management No. CDC-22, Decontamination of Laboratory Sink Drains to Remove Azide Salts” (Center for Disease Control, Atlanta, Georgia, April 30, 1976).

Limitations

Storage: The kit is temperature sensitive and must be stored at 2 - 8 °C.

Expiration: This test should be used within 18 months of purchase. Do not use reagents after the kit expiration.

IAA-tracer: Diluted IAA-tracer should be prepared as needed. Precise pipetting of the sample and IAA-tracer is critical to the accuracy and reproducibility of the assay.

Substrate: Dissolve PNP tablets completely before using.

PBST wash buffer: Once the PBST wash buffer has been diluted to the working concentration, azide should be added to make a 0.02 % solution if long term stability is desired.

IAA standard: Each standard strip can only be used once. The standard strip pad must be vortexed with TBS buffer to ensure that the standard is completely released from the pad and at the proper concentration. Use the standard immediately and do not store any remaining standard solution. It is important that a standard curve be included in each test run.

Results: Test is not valid unless B_o reads greater than 0.750 O.D. If the value is below this, increase the substrate incubation time until the desired O.D. is obtained (not to exceed 30 additional minutes).

Sample Preparations

Sample preparation procedures may vary with different types of plant materials. Results may be influenced by compounds such as terpenoids, phenolics, pigments or other plant components. Review the pertinent literature to determine whether extraction protocols have been established for the species of interest.

It is very important to methylate the sample extract prior to testing. A common method for methylation is via diazomethane or trimethylsilydiazomethane (Sigma Cat. #362832). Samples then require dilution in TBS buffer. It is important that the final extract contain no more that 10 % organic solvent in TBS buffer.

References

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Weiler, E. W. (1984) Immunoassay of plant growth regulators. Ann. Rev. Plant Physiol. 35:85-95.

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Weiler, E. W. (1982) An enzyme-immunoassay for *cis*-(+)-Abscisic Acid. Physiol. Plant. 54:510-514.

Weiler, E. W. (1981) Radioimmunoassay for pmol-quantities of Indole-3-acetic acid for use with highly stable [^{125}I]-and [^{3}H] IAA derivatives as radiotracers. Planta 153:319-325.

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Weiler, E. W. (1980) Radioimmunoassays for *trans*-zeatin and related cytokinins. Planta 149:155-162.

Weiler, E. W. (1980) Radioimmunoassays for the differential and direct analysis of free and conjugated Abscisic Acid in plant extracts. Planta 148:262-272.

Weiler, E. W. (1979) Radioimmunoassays for the determination of free and conjugated Abscisic Acid. Planta 144:255-263.

Prepare Buffers

The TBS buffer and PBST wash buffer are concentrated and must be diluted prior to use. Prepare only as much as will be needed for one day. Mix thoroughly, stirring each buffer for 15 to 30 minutes.

To prepare 100 mL of 1X TBS buffer, mix 5 mL of 20X TBS buffer with 95 mL of distilled water.

Prepare PBST wash buffer by diluting one 20X pouch of PBST wash buffer with 950 mL of distilled water.

Buffer formulations on page 7 are for reference only.

Directions for use

1. Prepare tracer solution:

Note: Standards and samples should be run in duplicate. Diluted IAA-tracer should be prepared as needed.

Dilute the IAA tracer with TBS buffer to a ratio of 1 to 400. In other words, for every mL TBS, add 2.5 µL of tracer. Mix the diluted solution well. Discard remaining diluted tracer solution after use.

2. Each standard strip can only be used once. Standard strips will make a standard solution that should be used within 6 hours then discarded. See the certificate of analysis for exact elution volume and concentration.

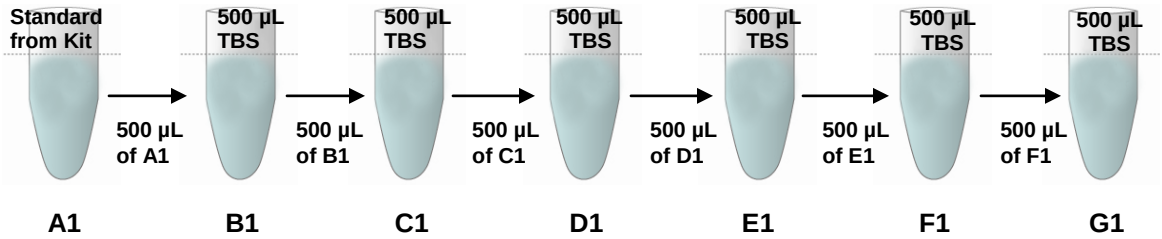
- Remove the pouch of hormone standards from the refrigerator and let them warm to room temperature.
- Remove a strip from the pouch and reseal the pouch with the desiccant. Hold the strip by the handle with the Agdia label; do not touch the filter paper at the bottom of the strip. Cut the filter paper at the arrows so that the filter paper falls into a microcentrifuge tube. Use forceps if necessary. The filter should fit inside the microcentrifuge tube so that the lid may close.
- Dispense 1.00 mL of 1X TBS buffer. Close the tube and vortex the solution with the filter for 30 seconds.
- Incubate the filter in the tube for 5 minutes, then vortex the solution for an additional 30 seconds. The solution approximately contains 5000 picomoles/mL (nM) IAA.

3. Following the chart below, prepare the other standards by diluting the standard solution further in 1X TBS buffer. New standards should be prepared each time the test is run.

Stock Solution (SS) = 1,000 picomoles/mL, NSB=Nonspecific Binding, B_o=100 % Binding

IAA Standard 09346
Note: Mix each dilution well

Plate Position	IAA Solution	1X TBS Buffer	[IAA] picomoles/mL	Dilution
A1= NSB	Standard; One strip		5,000	
B1	500 µL of A1	+500 µL	2,500	1:2
C1	500 µL of B1	+500 µL	1,250	1:2
D1	500 µL of C1	+500 µL	625	1:2
E1	500 µL of D1	+500 µL	312	1:2
F1	500 µL of E1	+500 µL	156	1:2
G1	500 µL of F1	+500 µL	78	1:2
H1=B_o	0	100 µL	0	



4. Remove the desired number of testwells from the pouch and place them in the testwell holder. Reseal the pouch, making sure the desiccant is still present, and return it to the refrigerator.

5. Add 100 µL of standard or sample extract to each well. Standards and samples should be run in duplicate.

6. Add 100 µL diluted tracer prepared in step 1 to each well using a multichannel pipette. Make sure the tips do not touch the solutions in the well so that cross contamination does not occur.

7. Mix the contents by gently swirling the plate on the bench top. Place plate in a humid box (airtight plastic box lined with damp paper towel). Make sure the humid box has been precooled to 2 - 8 °C for 30 minutes before use.

8. Incubate testwells in the refrigerator at 2 - 8 °C for 3 hours. Place a humid box in the 37 °C oven to preheat the box for the substrate incubation.

9. Prior to the end of the incubation period, prepare the substrate solution: Dissolve 1 substrate tablet in 5 mL of substrate diluent. Please be sure that the substrate tablet is completely dissolved and mixed before use.

10. After the 3 hour incubation, remove the testwells from the refrigerator and expel the contents of the testwells into the sink. For efficient expelling of the samples, while squeezing the long sides of the frame to hold the testwells in place, use a quick flipping motion to empty the contents of the wells into a sink or waste container.

11. Fill wells completely with 1X PBST wash buffer, and then quickly empty them again. Repeat 2 times. Grasp the testwell holder upside down then firmly tap it on a paper towel to remove remaining wash solution.

12. Add 200 µL of substrate solution to each well using a multichannel pipette.

13. Place plate in a humid box and incubate at 37 °C for 60 minutes.

14. Read the absorbance values at 405 nm. Test is not valid unless B_o reads greater than 0.750 O.D. If the value is below this, increase the substrate incubation time until the desired O.D. is obtained (not to exceed 30 additional minutes).

Calculations

1. Calculate the means of the optical densities of duplicate standards or samples.

2. Calculate the % Binding for the standard and sample with the following equation:

Definition of Symbols

NSB = Well A1 = 0 % Binding
B_o= Well H1 = 100 % Binding
O.D. = Optical Density / Absorbance value

% Binding = (Standard or Sample **O.D**. - NSB **O.D.**) / (B_o **O.D.** - NSB **O.D.**). Multiply by 100 for percentage.

3. After % Bindings have been calculated, calculate the logit value for the % Binding of standards and samples. See the equation below. Calculate the natural log for each standard concentration. Plot the Logit values on the y-axis and the correlating standard concentrations (in natural log values) on the x-axis. Calculate the y-intercept and slope from the linear curve generated with the standard data.

Logit equation for standard and sample % Binding values:

Logit = Ln[% Binding/(100-(% Binding))]

4. Use the following equation for the calculation of samples IAA concentration:

[Sample Concentration] = $e^{\text{(logit-(y-intercept)) / slope)}}$

Percent (%) cross-reactivity

For monoclonal antibody IAA 17 – II –A1

Compound	Cross-Reactivity*
Indole-3-acetic acid	100
Indole-3-acetic acid (a)	0
Indole-3-acetone	5.2
Indole-3-propionic acid	0.5
Indole-3-butyric acid	1.3
Indole-3-acetaldehyde	0.14
Indole-3-acetaldehyde (a)	0.05
Indole-3-ethanol	0.04
Indole-3-glyoxylic acid	0.03
Indole-3-acetonitrile	1
Indole-3-pyruvic acid	0.08
Indole-3-lactic acid	0.5
Indole-3-acylic acid	5.5
Indole-3-aldehyde (a)	0.2
Indole-3-acetamide	1
Indole-3-acetylglycine	57.9
Indole-3-acetylalanine	1.5
Indole-3-acetylphenylalanine	0.6
Indole-3-acetyl, DL-aspartic acid	0.5
Indole-3-acetyl-myo-inositol ester**(a)	0.2
5-Hydroxindole-3-acetic acid	0.02
1-Naphthylacetic acid	0.1
2-Naphthylacetic acid	0.03
2,3-Dichlorophenoxyacetic acid	0.01
2,4-Dichlorophenoxyacetic acid	0.01
3,5-Dichlorophenoxyacetic acid	less than 0.01
Phenylacetic acid	0.01
Imidazoleacetic acid	0.01
Urocanic acid	0.05
L-Tryptophan	0.04
D-Tryptophan	0.1

*Cross-reactivities were determined from tracer displacement curves at 50 % displacement on molar basis. All cross-reactants were treated with excess diazomethane prior to analysis, except those cross-reactants marked (a).

**Cross-reaction calculated from antibody saturation analysis using freshly purified (5-^{3}H) IAA-myo-inositol (29 Ci/mmol, up to 1819 pmol per assay).

Buffer Formulations

Substrate Diluent

Dissolve in 800 mL distilled water:

Magnesium chloride	0.1 g
Sodium azide	0.2 g
Diethanolamine	97.0 mL

Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 mL with distilled water. Store at 2 - 8 °C.

TBS Buffer (1X)

Dissolve in 800 mL distilled water:

Trizma base	0.53 g
Trizma hydrochloride	3.25 g
Sodium chloride	5.84 g
Magnesium chloride hexahydrate	0.20 g
Sodium azide, optional*	0.20 g

Adjust pH to 7.5. Adjust final volume to 1000 mL with distilled water. Store at 2 - 8 °C.

*Add sodium azide if long term stability is needed for storing unused buffer.

Trizma is a trademark of Sigma-Aldrich Biotechnology

PBST Buffer (Wash Buffer) (1X)

Dissolve in distilled water to 1000 mL:

Sodium chloride	8.00 g
Sodium phosphate, dibasic (anhydrous)	1.15 g
Potassium phosphate, monobasic (anhydrous)	0.20 g
Potassium chloride	0.20 g
Tween-20	0.50 g
Sodium azide, optional*	0.20 g

Adjust pH to 7.4.

* Add sodium azide if long term stability is needed for storing unused buffer.

Date _____ Test _____

Test performed by _____

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
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