

# Phytodetek® ABA Test Kit

Competitive ELISA, for the quantitative determination of Abscisic Acid  
Catalog number: PDK 09347/0096

## List of contents

| Lot number | Item  | 96 wells                  |
|------------|---|---------------------------|
| _____      | Hormone standard strips, ABA                              | 3 strips                  |
| _____      | Anti-ABA coated testwells                                 | 96 testwells              |
| _____      | ABA-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 ( <i>contains 0.02 % sodium azide</i> ) | 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. The Abscisic Acid (ABA) test utilizes an anti-ABA monoclonal antibody and is sensitive in the range of 0.032 – 0.16 picomoles ABA/mL.

The assay principle uses the competitive antibody binding method to measure concentrations of ABA in plant extracts. ABA-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 ABA-tracer, a limited amount of the antibody and the unknown sample containing ABA.

The ABA in the sample competes with the ABA-tracer for antibody binding sites. The unbound ABA-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 ABA concentration by means of a standard curve.

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## Warnings

Phytodetek ABA 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.

**ABA-tracer:** Diluted ABA-tracer should be prepared as needed. Precise pipetting of the sample and ABA-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, sodium azide should be added to make a 0.02 % solution if long term stability is desired.

**ABA 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  $B_0$  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 important that the final extract contain no more than 10 % organic solvent in TBS buffer. All samples require dilution in TBS buffer.

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## References

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- Weiler, E. W. (1982) An enzyme-immunoassay for *cis*-(+)-Abscisic Acid. *Physiol. Plant.* 54:510-514.
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- Weiler, E. W. and K. Spanier. (1981) Phytohormones in the formation of crown gall tumors. *Planta* 153:326-337.
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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 ABA-tracer should be prepared as needed.**

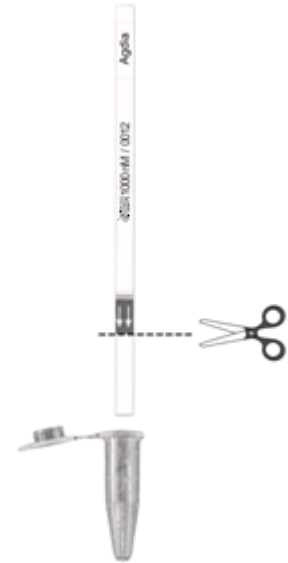
Dilute the ABA tracer with TBS buffer to a ratio of 1 to 400. In other words, for every mL of TBS, add 2.5 µL of tracer. Mix the diluted solution well. Discard remaining diluted tracer solution after use.

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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 700-1000 picomoles/mL (nM) (+)-ABA. See certificate of analysis for actual concentration. The kit recognizes only the (+)-ABA enantiomer.



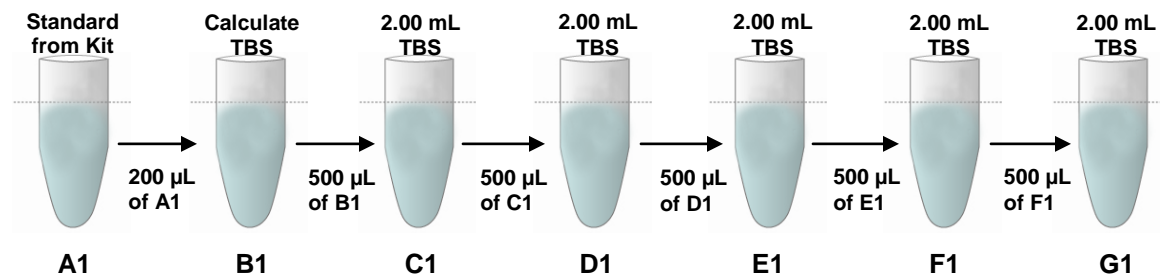
3. Following the chart below, prepare the other standards by diluting the standard solution further in 1X TBS buffer. New standards should be prepared each time the test is run.

**Stock Solution (SS) = 700 to 1000 picomoles/mL, NSB=Nonspecific Binding, B<sub>0</sub>=100 % Binding.**

Use the actual concentration of the eluted standard A1 from the certificate of analysis to calculate the dilution necessary to create standard B1 at exactly 100 picomoles/mL. For example, if A1 is 883 picomoles/mL, then dilute a 200 µL aliquot of A1 with 1.566 mL of TBS buffer to create standard B1 at 100 picomoles/mL.

**ABA Standard 09347** Note: Mix each dilution well.

| Plate Position    | ABA Solution        | 1X TBS Buffer | [ABA] picomoles/mL | Dilution    |
|-------------------|---------------------|---------------|--------------------|-------------|
| A1= NSB           | Standard; One strip |               | 700 – 1000         |             |
| B1                | 200 µL of A1        | (Calculate)   | 100                | (Calculate) |
| C1                | 500 µL of B1        | +2.00 mL      | 20                 | 1:5         |
| D1                | 500 µL of C1        | +2.00 mL      | 4                  | 1:5         |
| E1                | 500 µL of D1        | +2.00 mL      | 0.8                | 1:5         |
| F1                | 500 µL of E1        | +2.00 mL      | 0.16               | 1:5         |
| G1                | 500 µL of F1        | +2.00 mL      | 0.032              | 1:5         |
| H1=B <sub>0</sub> | 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.

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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).
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<sub>0</sub> 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<sub>0</sub> = Well H1 = 100 % Binding.**

**O.D. = Optical Density / Absorbance value**

% Binding =  $\frac{(\text{Standard or Sample O.D.} - \text{NSB O.D.})}{(\text{B}_0 \text{ O.D.} - \text{NSB O.D.})}$ . 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:

$$\text{Logit} = \ln\left[\frac{\% \text{ Binding}}{100 - (\% \text{ Binding})}\right]$$

4. Use the following equation for the calculation of samples ABA concentration:  
[Sample Concentration] =  $e^{(\text{logit} - \text{y-intercept}) / \text{slope}}$

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## Percent (%) cross-reactivity

For monoclonal antibody ABA - 15 - I - C - 5

| Compound   | Cross-Reactivity* |
|--|-------------------|
| 2- <i>cis</i> -(S)-ABA                                   | 100               |
| 2- <i>cis</i> -(S)-ABA methylester                       | Less than 0.1     |
| 2- <i>cis</i> -(R)-ABA                                   | 0                 |
| 2- <i>trans</i> -(S)-ABA                                 | 0                 |
| 2- <i>cis</i> -(S)-ABA- <i>B</i> -D-glucopyranosyl ester | 0                 |
| 2- <i>cis</i> -(S)-ABA- <i>cis</i> -diol                 | 0                 |
| Phaseic acid   | Less than 0.1     |
| Dihydrophaseic acid                                      | Less than 0.1     |
| Xanthoxin  | 0                 |
| All- <i>trans</i> -Farnesol                              | 0                 |

\*Cross-reactivities were determined from tracer displacement curves at 50 % displacement on molar basis.

**Important Note:** If you are trying to determine the concentration of *cis* (+) ABA when using *cis/trans* (+/-) ABA as an analytical standard, the effective concentration of *cis* ABA is one half the value of *cis/trans* ABA. For example: 10 picomoles *cis/trans* ABA is 5 picomoles *cis* ABA.

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## 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 you will need long term stability for storing unused buffer.

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### 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 you will need long term stability for storing unused buffer.