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Product Information

Plant Growth Regulator Immunoassay Detection Kits

Catalog Number **PGR1**, Abscisic Acid
Catalog Number **PGR3**, Indole-3-Acetic Acid
Catalog Number **PGR4**, Isopentenyl Adenosine
Catalog Number **PGR5**, *trans*-Zeatin Riboside

Storage Temperature 2–8 °C

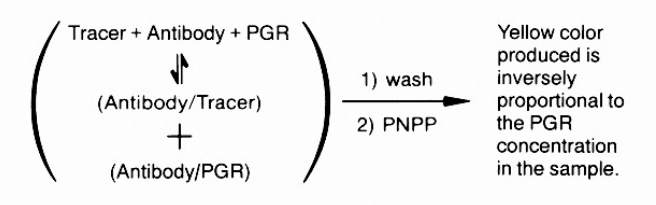
TECHNICAL BULLETIN

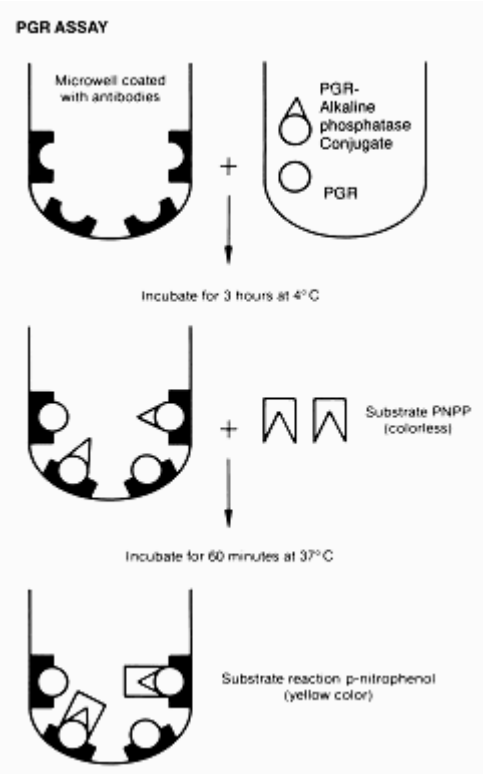
Product Description

The plant growth regulator (PGR) enzyme immunoassay detection kits are convenient tests for the quantitative determination of plant hormones. The tests utilize monoclonal antibodies specific to the targeted hormones.

The assay principle uses the competitive antibody binding method to measure concentrations of PGR in 0.1 mL of plant extracts. Each kit provides the relevant PGR-alkaline phosphatase tracer, which is added along with the plant extract to the antibody coated microwells. A competitive binding reaction is set up between a constant amount of the tracer, a limited amount of the antibody, and the unknown amount of PGR in the plant extract.

The hormone in the sample competes with the tracer for antibody binding sites. The unbound tracer is washed away before adding the substrate, *p*-nitrophenylphosphate (PNPP). Hence, the yellow color produced when the enzyme of the tracer reacts with PNPP to produce *p*-nitrophenol is inversely proportional to the amount of hormone in the sample. The intensity of color is related to the PGR concentration in the sample by means of a standard curve.





The indole-3-acetic acid (IAA) test (PGR-3) uses a monoclonal antibody to the IAA-methyl ester. Consequently, to detect IAA in the plant extract, the sample must first be treated with diazomethane to convert the hormone to its methyl ester form. The standard included in the PGR-3 kit is indole-3-acetic acid methyl ester.

The sensitivity range for PGR-3 (IAA) is 0.5–100 pmole/0.1 mL. All other kits have a sensitivity range of 0.02–0.5 pmole/0.1 mL.

Components

Catalog Number	Description	Multiwell Plate	Plate Sealer	Tracer	Substrate Diluent*	Wash Solution, 20×*	TBS, 20×	Substrate (PNPP)
		1 each	2 each	3 × 1 mL	30 mL	3 × 50 mL	60 mL	6 tablets
PGR1	Abscisic acid	M7537	P3200	T7916	S2154	W1629	T8541	S2029
PGR3	Indole-3-acetic Acid	M7662	P3200	T8041	S2154	W1629	T8541	S2029
PGR4	Isopentenyl Adenosine	M7787	P3200	T8166	S2154	W1629	T8541	S2029
PGR5	*trans*-Zeatin Riboside	M8037	P3200	T8416	S2154	W1629	T8541	S2029

* Contain 0.02% sodium azide

Reagents and Equipment Required but Not Provided

- Microplate reader fitted with 405 nm filter
- Transfer Pipettes
  - 1 mL volumetric
  - 5 mL serologic
  - 100 µL single channel
  - 50–200 µL multichannel
- Plant Growth Regulator (PGR) Standards:
  - Isopentenyl adenosine - [(Dimethylallylamino)-purine riboside]
  - *trans*-Zeatin riboside, Catalog Number Z3541
- Absolute methanol
- Distilled water
- Test tubes
- Pipette tips
- Timer
- Reservoirs for substrate, wash, tracer, and stop solutions
- 37 °C Incubator – Forced air microplate incubator recommended
- Refrigerator

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Note: Read Instructions Completely Before Using These Kits. The entire procedure should be carefully reviewed before starting the assay.

Preparation Instructions

Procedures for sample preparation (i.e., extracting PGRs from plant tissue) are not provided. The user of this kit is encouraged to review the scientific literature for appropriate protocols, which apply to the specific PGR(s) and plant tissue(s) of interest.

Tracer Solution – Add 5 mL of 1× TBS to the tracer vial and allow 5 minutes for complete reconstitution. Invert bottle several times to ensure proper mixing. Each tracer vial contains sufficient material to perform 32 assays (4 strips). Tracer Solution can be stored at 2–8 °C for up to 7 days.

Substrate Solution – Dissolve one substrate tablet in 5 mL of Substrate Diluent. One tablet is sufficient to perform 16 assays (2 strips).

Standard Solutions – Make the following stock solutions by dissolving the PGR standard in absolute methanol:

1.0 mM PGR-1
10.0 mM PGR-3
0.1 mM PGR-4
0.1 mM PGR-5

From these stock solutions make standards in 25 mM Tris buffered saline, pH 7.5. For all kits, ***except PGR-3***, a minimum of 4 points at 0.05, 0.1, 0.5, and 2.0 pmole/0.1 mL along with a 100 pmole/0.1 mL ($B_{0\%}$) and 0 pmole/0.1 mL (blank = $B_{100\%}$) standards are recommended for the standard curve. The sensitivity is optimal between 0.02–5.0 pmole/0.1 mL.

For **PGR-3** a minimum of 4 points at 1, 5, 20, 50 pmole/0.1 mL along with a 500 pmole/0.1 mL ($B_{0\%}$) and 0 pmole/0.1 mL (blank = $B_{100\%}$) standards are recommended for the standard curve. The sensitivity of PGR-3 is optimal between 0.5–100.0 pmole/0.1 mL.

Storage/Stability

Store the kits at 2–8 °C. Do not use reagents after their expiration dates. All reagents should be stored as indicated on the label.

Tracer solution remains active for 7 days at 2–8 °C. Substrate Solution remains active for 8 hours at 2–8 °C.

Procedure

Precise pipetting of sample and tracer solutions are critical to the accuracy and reproducibility of the assay. It is important that a standard curve be included in *each run* when all strips are not processed at the same time.

1. Remove desired number of strips from refrigerator and place in strip holder. Add 100 µL of standard or sample solution to each well.
   Note: When using the **PGR-3** kit, samples should be treated with diazomethane (to convert free indoleacetic acid to the methyl ester).
2. Add 100 µL of Tracer Solution to each well using a multichannel pipette. Mix by tapping plate. Cover wells with plate sealer.
3. Incubate sealed wells in refrigerator at 2–8 °C for three hours. After the 3 hour incubation, remove the strips from the refrigerator and decant solution.
4. Wash wells by adding 200 µL of the Wash Solution to each well with a multichannel pipette. Decant wash mixture from the wells. Firmly squeeze raised tabs along horizontal edge (wells 5–8) while decanting to prevent loss of strips. Repeat this step 2 more times and remove excess Wash Solution by patting strips dry on paper towels.
5. Add 200 µL of Substrate Solution to each well using a multichannel pipette. Mix by tapping plate. Cover wells with plate sealer.
6. Incubate at 37 °C for 60 minutes. Remove strips from incubator.
   Note: Test is not valid unless $B_{100\%}$ reads >0.750 OD. If the value is below this, increase the substrate incubation time until the desired OD is obtained (not to exceed 30 additional minutes).
7. Record optical densities at 405 nm.

Results

Calculations

1. Record the optical densities.
2. Average the optical densities of duplicate standards or samples.
3. Calculate the % Binding of each standard point or sample by the following:

$$\%\ Binding\ (B\%) = \left(\frac{Standard\ or\ Sample\ O.D. - B_{0\%}\ O.D.}{B_{100\%}\ O.D. - B_{0\%}\ O.D.} \right) X\ 100$$

$B_{100\%}$ (100% Binding) =
100 µL buffer + 100 µL tracer

$B_{0\%}$ (Non-specific Binding, 0% Binding) =
100 pmole/assay PGR* + tracer =

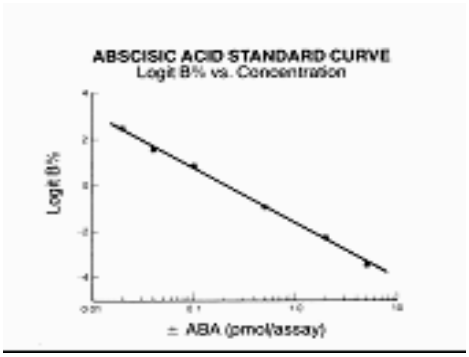
* When calculating results: If (±)PGR standard is used, then the effective standard concentration is one-half the indicated concentration. (Example: 10 pmole (±)PGR is in effect 5 pmole (+)PGR plus 5 pmole (–)PGR standard).

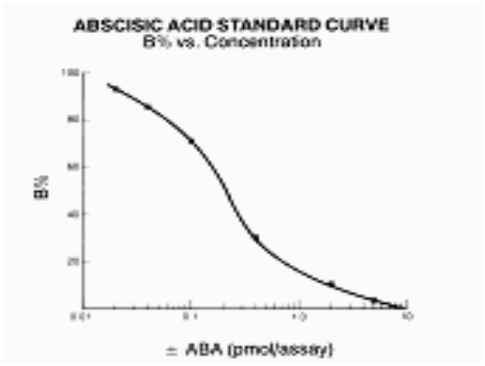
4. Plot the % Binding (B%) versus the log of the total concentration (pmole) of PGR* and draw the best fit curve. Semi-log paper can be used for convenience.
   Note: Linear relationship can be plotted by graphing logit B% vs log concentration.

$$Logit\ B\ /\ Bo = \mathrm{Ln}\left(\frac{B\ /\ Bo\%}{100 - B\ /Bo\%} \right)$$

5. The sample concentration is determined by extrapolation of the sample % Binding from the best fit standard curve.

Typical Calibration Curves





% Cross Reactivity

Determined from tracer displacement curves at 50% displacement on molar basis.

Cross Reactant ABA	**15-I-C_5**
2-*cis*-(S)-ABA	100.00
2-*cis*-(S)-ABA methylester	<0.10
2-*cis*-(R)-ABA	0.00
2-*trans*-(S)-ABA	0.00
2-*cis*-(S)-ABA-β-D-glucopyranosyl ester	0.00
2-*cis*-(S)-ABA-*cis*-diol	0.00
Phaseic acid	<0.10
Dihydrophaseic acid	<0.10
Xanthoxin	0.00
All-*trans*-Farnesol	0.00

Cross Reactant IPA	**J40-IV-C4**
Isopentenyl Adenosine	100.00
Dihydrozeatin	0.50
Dihydrozeatin Riboside	0.70
cis-Zeatin	0.01
cis-Zeatin Riboside	0.01
trans-Zeatin	0.30
trans-Zeatin Riboside	0.40
Isopentenyl Adenine	53.10
Dihydrozeatin-O-glucoside	0.00
Dihydrozeatin Riboside-O-glucoside	0.00
Zeatin-O-glucoside	0.00
Zeatin Riboside-O-glucoside	0.00
6-Furfurylaminopurine (Kinetin)	0.10
6-*n*-Hexylaminopurine	<0.01
6-Benzylaminopurine-9-glucoside	0.08
6-Benzylaminopurine-7-glucoside	0.00
6-Benzylaminopurine-3-glucoside	0.00
6-Amino-3-Dimethylallylpurine	0.20
Adenine	0.00
Adenosine	0.00
Guanine	0.00
Guanosine-5′-triphosphate	0.00
Cytosine	0.00
Cytidine	0.00
Inosin-5′-triphosphate	0.02
6-Piperidino-1-purine	<0.01
4-(2-Ethylhexyamino)-2-methylpyrrolo (2,3-d)-pyrimidine	0.00
4-Isobutylamino-2-methylpyrrolo (2,3-d)-pyrimidine	0.00
4-Allylamino--2-methylpyrrolo (2,3-d)-pyrimidine	0.00
4-(1,1-dimethyl-3-hydroxy-propylamino)-pyrimidine 2-methylpyrrolo (2,3-d)-pyrimidine	0.00
4-(3-hydroxpropylamino)-2-methylpyrrolo (2,3-d)-pyrimidine	0.00

Cross Reactant* IAA	17-II-A
Indole-3-acetic acid	100.00
Indole-3-acetic acid (a)	0.00
Indole-3-acetone	5.20
Indole-3-propionic acid	0.50
Indole-3-butyric acid	1.30
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	1.00
Indole-3-pyruvic acid	0.08
Indole-3-lactic acid	0.50
Indole-3-acrylic acid	5.50
Indole-3-aldehyde (a)	0.20
Indole-3-acetamide	1.00
Indole-3-acetylglycine	57.90
Indole-3-acetylalanine	1.50
Indole-3-acetylphenylalanine	0.60
Indole-3-acetyl, DL-aspartic acid	0.50
Indole-3-acetyl-*myo*-inositol ester** (a)	0.20
5-Hydroxyindole-3-acetic acid	0.02
1-Naphthylacetic acid	0.10
2-Naphthylacetic acid	0.03
2,3-Dichlorophenoxyacetic acid	0.01
2,4-Dichlorophenoxyacetic acid	0.01
3,5-Dichlorophenoxyacetic acid	<0.01
Phenylacetic acid	0.01
Imidazoleacetic acid	0.01
Urocanic acid	0.05
L-Tryptophan	0.04
D-Tryptophan	0.10

Cross Reactant T-ZR	J3-I-B_3
trans-Zeatin Riboside	100.00
Dihydrozeatin	2.30
DihydroZeatin Riboside	1.20
cis-Zeatin Riboside	0.80
cis-Zeatin	0.40
trans-Zeatin	47.30
Zeatin Riboside-5′-monophosphate	95.20
DihydroZeatin Riboside-O-glucoside	0.07
Dihydrozeatin-O-glucoside	0.80
Zeatin-O-glucoside	7.70
Zeatin Riboside-O-glucoside	0.80
Isopentenyl Adenosine	0.50
Isopentenyl Adenine	0.90
6-Furfurylaminopurine (Kinetin)	0.06
6-*n*-Hexylaminopurine	0.20
6-Benzylaminopurine-9-glucoside	0.70
6-Benzylaminopurine-7-glucoside	<0.01
6-Benzylaminopurine-3-glucoside	0.06
6-Amino-3-dimethylallylpurine	0.70
Adenosine	<0.01
Adenine	<0.01
Guanine	<0.01
Guanosine-5′-triphosphate	<0.01
Cytosine	<0.01
Cytidine	0.00
Inosine-5′-triphosphate	0.01
6-Piperidino-1-purine	<0.01

* All cross reactants 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/mmole, up to 1819 pmole per assay).

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