PAT/Bar ELISA Kit

Cat No. AID 018

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Intended use

PAT/Bar ELISA test kit is intended to be used for the qualitative detection of Liberty Link PAT/Bar protein in individual leaf/seed samples. The kit detects phosphinothricin acetyl transferase enzyme (PAT) coded for by the Bar gene in leaf or seed. The kit can be used for canola, rice, cotton and mustard leaf or seed sample.

The total incubation time of the assay is 75 minutes. Liberty link PAT from the pat gene is not detected by this kit

Principle of the test

An antibody specific to the PAT/Bar protein molecule is immobilized on the micro well plates and second antibody specific for PAT/Bar molecule is conjugated with horse radish peroxidase (HRP).

When the sample extracts are added to the micro titer wells, the PAT/Bar protein from sample binds to the antibody immobilized in the well. This binding is subsequently detected by addition of enzyme labeled antibody. After a washing step, substrate is added. The enzymatic reaction and development of color is proportional to the amount of PAT/Bar protein present in the sample. The reaction is terminated by addition of stopping solution. Absorbance is measured on a plate reader.

Light color indicates lower concentration while dark color indicates higher concentration of PAT/Bar protein in the sample.

Cross-reactivity

The PAT/Bar ELISA kit does not recognize Cry1F, Cry2A, Cry9C, Cry1Ac/Ab or CP4EPSPS.

Precautions

The PAT/Bar ELISA test is intended for in vitro use only. The reagents contain Thimerosal as preservative. Prevent direct skin and eye contact with kit components. Seek medical attention in case of accidental ingestion of kit components

Storage of the kit

The kit should be stored at 2 - 8° C. The unopened kit is stable till the expiry date printed on the kit label. The expiry date of each unopened component is printed on the label of the component.

Contents of the kit

Kits are provided in 1, 10 or 50 plates format

Contents of one plate kit:

1. One plate of 96 wells coated with Anti-Pat/Bar antibody, packed in a laminate bag with silica gel
2. Pat/Bar enzyme conjugate: One Ready-to-use bottle of 6 ml
3. Extraction buffer: (10X Stock): 50 ml, One vial
4. Wash solution: (10X Stock): 50 ml, One vial
5. TMB Substrate, One Ready-to-use bottle of 12 ml
6. Pat/Bar Negative control: One ready-to-use vial of 2 ml

Contents of 10-plate kit

1. Ten plates of 96 wells coated with Anti-Pat/Bar antibody, individually packed in a laminate bag with silica gel
2. Pat/Bar enzyme conjugate: One Ready- to- use bottle of 55 ml
3. Extraction buffer concentrate: One packet of powder with a 25 ml vial of Tween-20
4. Wash solution concentrate: One packet of powder with a 2.5 ml vial of Tween-20
5. TMB Substrate: One ready-to-use bottle of 110 ml
6. Pat/Bar Negative control: One ready-to-use vial of 10 ml

Contents of 50-plate kit

1. Fifty plates of 96 wells coated with Anti-Pat/Bar antibody, each packed individually in a laminate bag with silica gel
2. Pat/Bar enzyme conjugate: One ready to use bottle with 275 ml.
3. Extraction buffer concentrate: One packet of powder with a 25 ml vial of Tween-20
4. Wash solution concentrate: One packet of powder with a 2.5 ml vial of Tween-20
5. TMB Substrate: One ready-to-use bottle of 500 ml
6. Pat/Bar Negative control: One ready-to-use vial of 10 ml

Material and equipment required but not provided

- Pipette with disposable plastic tips
- Multi channel pipette with disposable pipette tips
- Deionized or distilled water
- Graduated cylinders with one liter capacity
- Reagent troughs
- Plate ELISA washer or wash bottle
- Plate ELISA reader with 450/620 nm filter
- Table top centrifuge
- Marking pen, Parafilm*, Pipette tips, Timer and Paper towels

Reagent preparation

The procedure for preparation of reagent is as follows

For one plate kit

Preparation of working wash buffer:

To 450 ml of distilled/deionized water, empty entire contents of the extraction buffer vial. Mix well before use. Store unused portion at 2 - 8° C.

Preparation of working extraction buffer:

To 450 ml of distilled/deionized water, empty entire contents of the wash buffer vial. Mix well before use. Store unused portion at 2 - 8° C.

For 10 and 50 plate kit

Preparation of working extraction buffer:

Dissolve the packet of extraction buffer concentrate powder in 500 ml of distilled/deionised water and add 25 ml of Tween-20 which is provided along with the powder. Mix it well before use. The resultant extraction solution is 10X concentrate.

This has to be further diluted with 4500 ml of water to have a working extraction buffer.

Store unused portion of working extraction buffer at 2 - 8°C. Thaw to room temperature before using the same again.

Preparation of Working wash buffer:

Dissolve the packet of wash buffer concentrate powder in 500 ml of distilled/deionised water and add 2.5 ml of Tween-20 which is provided along with the powder. Mix it well before use. The resultant wash solution is 10X concentrate.

This needs to be further diluted with 4500 ml of water to have working wash buffer

Store unused portion of working wash buffer at 2 - 8° C. Thaw to room temperature before using the same again.

Preparation of stop solution:

Add 27.0 ml of 98 % H_2SO_4 in 973 ml of deionized or distilled water. Work in fume hood while preparing stop solution. Store at room temperature for up to one year.

Preparation of user positive control:

The user is advised to prepare in-house positive control. Crush or grind one known positive seed into a uniform powder and add 1.0 ml of extraction buffer. Wait for 10 minutes before use. Prepare fresh before use.

Sample preparation:

Individual seed:

Crush or grind seed thoroughly into a uniform powder using mortar and grinder or seed crusher. Add 1.0 ml of extraction buffer to each sample. Allow the mixture to stand for 30 minutes at room temperature (RT) on a shaker. Allow particle to settle and use only the supernatant to do the test. Wash and rinse grinding equipment carefully between samples to avoid cross contamination.

Individual leaf:

Weigh (two fresh leaf punch, about 20 mg) and add it to a microtube. Crush leaf with pestle in 0.5 ml extraction buffer. Mix well and incubate for 30 minutes at RT on a shaker. Centrifuge at 3000 rpm for 10 minutes and use only the supernatant to do the test. Avoid cross contamination between samples.

Assay Procedure

Test protocol for individual seed or leaf samples. Estimated procedure time is 60 minutes)

1. Allow all reagents to reach room temperature before use.
2. Add 50 µl enzyme conjugate to each well.
   Add 50 µl working extraction buffer to blank well. Add 50 µl of negative control in two wells Add 50 µl of sample extracts to wells. Mix contents of the plate carefully, to avoid cross contamination.
3. Cover the plate and incubate it for 60 minutes at room temperature.
4. Remove content of the wells by decanting into a sink or a waste container. Add 300 µl/well wash solution to all wells and then empty wells by inverting the plate. Repeat washing three more times. Alternately, perform four washes by using microtiter plate washer. After last wash, tap the inverted microtiter plate on paper towel to remove as much liquid as possible.
5. Add 100 µl substrate per well.
6. Cover the plate and incubate for 15 minutes at room temperature.
7. Stop the reaction by adding 100 µl stop solution.
8. Measure the absorbance at 450 nm (primary filter) and 620/630 nm (secondary filter). Read the plate within 15 minutes after addition of stop solution.

Notes on technique:

1. Protect the plates from draught, strong light or direct sunlight during the test procedure.
2. Careful aspiration of the washing solution is essential for good assay precision.
3. Since the timing of the incubation steps is important to performance of the assay, use multi channel pipettes to dispense the sample/reagents.
4. Plate readers measure absorbance vertically. Do not touch the bottom of the wells

Interpretation of results:

General test criteria:

- Absorbance of the blank well should not exceed 0.15
- Absorbance value of blank should be subtracted from positive control, negative control and samples.
- The coefficient of variance between the duplicate positive controls should be less than 15 %
- Mean negative control should have absorbance below 0.1

If above criteria are not met, the test is invalid and should be repeated.

Qualitative test

For single canola, rice, cotton and mustard leaf or seed samples:

Cutoff value = (Mean absorbance of negative control + 0.2)

Positive or Negative Sample

Individual seed or leaf samples are either positive or negative.

Positive sample: If sample absorbance is more than the cutoff value.

Negative sample: If sample absorbance is less than the cutoff value.

Low level results normally indicate improper washing, cross contamination or technique. In such cases, retesting is recommended.

Performance characteristics

Sensitivity: 100 %

Specificity: 100 %

WARRANTY

Eurofins Amar Immunodiagnostics Pvt. Ltd. warrants that the products sold hereunder ("the Product") are defect-free in material and workmanship, provided they are used in accordance with the prescribed instructions before the expiry of the products as printed on the product label.

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