

Rapid lateral flow strip (LFS) kit for Simultaneous Trait Detection of Bollgard II (Cry1Ac, Cry2A) in cotton seed and leaf

Cat No. AID 038



Technologies

Intended use

The kit is used for the qualitative detection (yes/no) of Cry1Ac and Cry2A Proteins present in Bollgard II in genetically modified individual cotton seed or leaf.

The total incubation time of the test is 10 minutes.

Principle of the test

An antibody specific to Cry1Ac and Cry2A immobilized on the nitrocellulose membrane as two different test lines. An anti-mouse IgG is immobilized as control line, Second antibody specific for Cry1Ac and Cry2A is conjugated with colloidal gold and incorporated in the sample area of lateral flow strip.

When the LFS is placed in the sample extract, the Cry1Ac or Cry2A protein, if present in the sample extracts binds to the corresponding antibody labelled with gold nano particles and the complex moves upward by capillary action. The complex then binds to the antibody coated on the membrane as two different test lines resulting in pink/purple color test line. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In absence of protein in sample extract (Cry1Ac or Cry2A) the corresponding test line does not appear. Control line always appears in pink/purple color indicating validity of the test protocol.



Punching a leaf in a microfuge vial

Contents of the kit: Kit is sufficient for 100 tests

Cry1Ac or Cry2A strips:	50 strips in one canister. Two canisters per kit
Extraction buffer	Two vials of 50 ml each. Ready to use
Dropper	One
Pack insert	One

Material required but not provided

- ❖ 1.5 ml Microfuge tubes with cap
- ❖ Hammer
- ❖ Timer
- ❖ 48 well seed crushing plate
- ❖ Pair of scissors
- ❖ Pestles
- ❖ 48 prong seed crusher
- ❖ Pliers
- ❖ Pen Marker and paper towels

Precautions

The Cry1Ac or Cry2A LFS kit is intended for in vitro use only. The reagents contain sodium azide 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. Exposure to moisture is likely to affect the performance of the test strips.

The kit should not be frozen.

Sample preparation

Extraction of seed tissue:

Crush single seed and transfer it to 1.5ml microfuge tube. Add 1.0 ml of extraction buffer. Mix well and wait for five minutes.

Or

Use 48 well seed crushing plate, seed crusher and hammer to crush 48 seeds at a time. Add 1.0ml of extraction buffer using calibrated dropper. Mix well and wait for 5 minutes.

Extraction of leaf tissue:

Take two leaf punch (approximate weight 20 mg) and transfer it to 1.5 ml microfuge tube. Grind the leaf tissue with pestle until the leaf tissue is well ground. Add 0.5 ml extraction buffer. Mix well and wait for 5 minutes. Use new pestle for each leaf sample to avoid cross contamination.

Crushed seeds should be tested on the same day.




Extracting protein by crushing with pestle

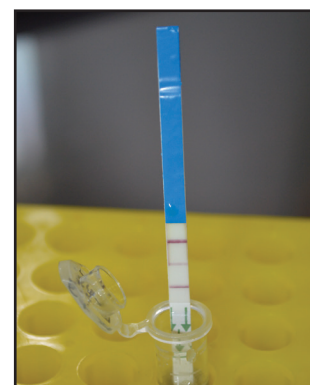
Assay Procedure

1. Allow canister to come to room temperature before opening it to remove the desired number of strips.
2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the microfuge tube/Crushing plate vertical position for 10 minutes.
3. Remove the strip and observe the result. Positive sample result may appear much earlier than 5 minutes.
4. For permanent storage of strips, cut off the bottom section of the strip covered with arrow using pair of scissors.

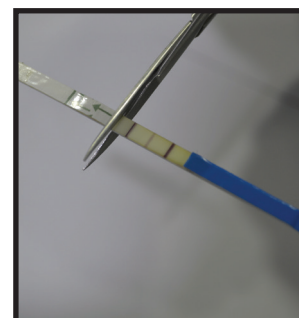
Interpretation guide:



Interpretation Guide				
	+Cry1Ac, +Cry2A	-Cry1Ac, +Cry2A	+Cry1Ac, -Cry2A	-Cry1Ac, -Cry2A
Control Line	—	—	—	—
Cry2A Test Line	—	—	—	—
Cry1Ac Test Line	—	—	—	—



Strip in leaf extract with positive result



Cutting of strip for permanent record and storage

Interpretation of LFS results:

Read the strip in 10 minutes.

Presence of Control line:

Control line should always be present. Absence of control line makes test invalid and the test should be repeated.

Control line will appear below the absorbent pad as per the picture above.

Presence of Test lines:

The **second line** from top is for Cry2A and it will appear if the sample is positive for Cry2A.

The **third line** from the top is for Cry1Ac and it will appear if the sample is positive for Cry1Ac.

Notes

- ❖ The procedure instructions should be strictly followed to get correct results. Change in procedure may lead to wrong results.
- ❖ This kit has been optimized for individual Cotton seed and leaf.
- ❖ Cross contamination between different wells/microfuge tubes will lead to wrong results
- ❖ This kit is meant for screening for presence or absence of Cry1Ac or Cry2A in individual Cotton seed and leaf. When in doubt, please confirm results with an alternate method.

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.

The customer should notify Eurofins Amar Immunodiagnostics in writing of Warranty defects during the warranty period, including an offer by the customer to return the Products to Eurofins Amar Immunodiagnostics for evaluation. Eurofins Amar Immunodiagnostics will repair or replace, at its sole option, any product or part thereof that proves defective in materials or workmanship within the warranty period.

This warranty also does not apply to Products to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Eurofins Amar Immunodiagnostics

THIS WARRANTY IS EXCLUSIVE

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***Liberty Link is a register trademark of Bayer Crop Science.

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