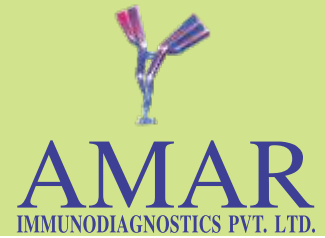


Rapid Lateral Flow Strip (LFS) kit for simultaneous trait Detection of Bollgard II (Cry1Ac, Cry2A) in cotton seed and leaf Cat No. AID 038



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:

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

When the LFS is placed in the cotton seed or leaf extract, the Cry1Ac or Cry2A proteins, if present in the sample extract bind to the corresponding antibody labelled with gold 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 colour test lines. As the complex moves further up, it binds to the control line resulting in pink/purple colour control line. In the absence of protein in sample extract (Cry1Ac or Cry2A), the corresponding test line does not appear. Control line always appears in pink/purple colour indicating validity of the test protocol.



Punching a leaf in microfuge vial

Contents of the kit: Kit is sufficient for 100 tests.

Cry1Ac/Cry2ALFS kit:	50 strips in one canister. Two canisters per kit
Extraction buffer:	Two bottles each with 50 ml extraction buffer
Pack insert:	One
Dropper:	One

Note: Kit is also available in 500, 1000 and 5000 test pack.

Material required but not provided

*1.5 ml Microfuge tubes with cap	* 48 well seed crushing plate	* Marking pen
* 48 prong seed crusher	* Hammer	* Pestles
* Pliers	*Timer	*Pair of scissors
* 5 ml plastic tubes		

Precautions

The Cry1Ac/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 can be stored at 25° C or below for short duration of 1-2 days. The kit should not be frozen.

Sample preparation:

Extraction of seed tissue:

Crush single cotton seed with pliers and transfer seed powder to 5.0 ml tube. Add 1.0 ml of extraction buffer using calibrated dropper. Mix well and wait for 5 minutes.

or

Use 48 well seed crushing plate, seed crusher and hammer to crush 48 seeds at a time. Add 1.0 ml of extraction buffer using calibrated dropper. Mix well and wait for 10 minutes.

Crushed seeds should be tested on the same day.



Extracting protein by crushing with pestle

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.

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/plastic tubes in vertical position for 10 minutes.
3. Remove the strips and observe the results. Positive sample results may appear much earlier than 10 minutes.
4. For permanent storage of strips, cut off the bottom section of the strip covered with arrow using pair of scissors.



Strip in leaf extract with positive results

Interpretation of LFS results:

Read the strip in 10 minutes.

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

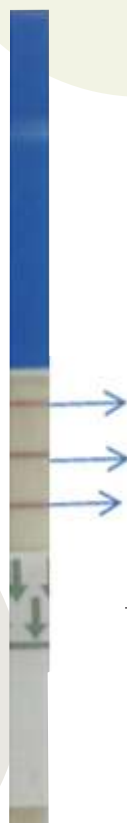
Presence of only one line:

If the sample is negative for Cry1Ac and Cry2A, only control line will appear below the absorbent pad as per the picture below.

Presence of two to three 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.



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

— indicates where a line will appear on the strip

Notes:

- ❖ The appearance of faint test line after 10 minutes should not be necessarily interpreted as positive test.
- ❖ 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 or leaf.
- ❖ When in doubt, please confirm results with an alternate method.

WARRANTY

Amar Immunodiagnostics Pvt Ltd warrants that the products sold hereunder ("the Products") 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 Amar Immunodiagnostics in writing of warranty defects during the warranty period, including an offer by the customer to return the products to Amar Immunodiagnostics for evaluation. Amar Immunodiagnostics will repair or replace, at its own option, any product or part thereof that proves defective in material 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 Amar Immunodiagnostics

THIS WARRANTY IS EXCLUSIVE

The sole and exclusive obligation of Amar Immunodiagnostics shall be to repair or replace the defective products in the manner and for the period provided above.

Amar Immunodiagnostics shall not have any other obligation or liability, whatsoever it may be, with respect to the products or any part thereof. Under no circumstances, whatsoever the circumstances may be, shall Amar Immunodiagnostics be liable for accidental, special or consequential damages. If any part of this warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

*Bollgard , Bollgard II and RoundUp are registered trade marks of Monsanto Company.

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