

Cat No. AID 035

Intended use

The kit is used for the qualitative detection (yes/no) of Cry1Ac, Cry2A and CP4 EPSPS proteins present in Bollgard II and Bollgard II plus Roundup Ready 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, Cry2A and CP4EPSPS are immobilized on the nitrocellulose membrane as three different test lines. An anti-mouse IgG is immobilized as control line. Second antibody specific for each Cry1Ac, Cry2A and CP4EPSPS 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, Cry2A or CP4EPSPS proteins, if present in the sample extract bind 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 three different test lines resulting in pink/purple color test lines. As the complex moves further up, it binds to the control line resulting in pink/purple color control line. In the absence of protein in sample extract (Cry1Ac, Cry2A or CP4EPSPS), the corresponding test line does not appear. Control line always appears in pink to purple color indicating validity of the test protocol.

Contents of the kit: Kit is sufficient for 100 tests

Cry1Ac/Cry2A/CP4(RR) LFS strips: 50 strips in one canister. Two canisters per kit

Extraction buffer: Two vials of 50 ml each. Ready to use

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
- ❖ 48 prong seed crusher
- ❖ Hammer
- ❖ Pliers
- ❖ Timer
- ❖ Marking pen and paper towels
- ❖ Pestles
- ❖ Pair of scissors

Precautions

The Cry1Ac/Cry2A/CP4EPSPS 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. The cap of the canister should be closed firmly after removing the required strips. 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 cotton seed with pliers and transfer seed powder to 1.5 ml microfuge 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 5 minutes.

Crushed seeds should be tested on the same day.



Punching a leaf in microfuge vial



Extracting protein by crushing with pestle


Extraction of leaf tissue:

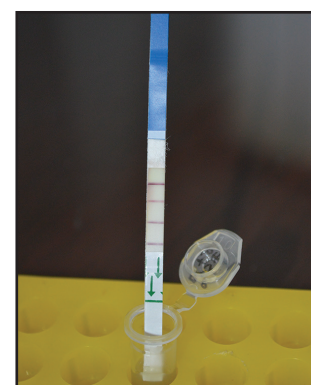
Take two leaf punch (approximate weight 20 mg) and transfer it to 1.5 ml microfuge tube. Grind the leaf tissue with pestle until the leaf tissue is well ground. Add 0.5 ml extraction buffer using calibrated dropper. Mix well and wait for 5 minutes. Use new pestle for each leaf sample to avoid cross contamination.

Assay Procedure

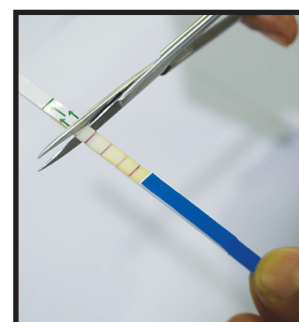
1. Allow canister to come to room temperature before opening it to remove the desired number of strips.
2. Insert one strip in each sample. Part of the strip showing arrow should be dipped in sample extract. Allow the test strip to remain in the seed crushing plate/microfuge tube in vertical position 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.

Interpretation guide:

		Interpretation Guide							
		+RR, +Cry1Ac, +Cry2A	-RR, +Cry1Ac, +Cry2A	+RR, -Cry1Ac, +Cry2A	+RR, +Cry1Ac, -Cry2A	-RR, -Cry1Ac, +Cry2A	+RR, -Cry1Ac, -Cry2A	-RR, +Cry1Ac, -Cry2A	-RR, -Cry1Ac, -Cry2A
	Control Line	—	—	—	—	—	—	—	—
	Cry2A Test Line	—	—	—	—	—	—	—	—
	Cry1Ac Test Line	—	—	—	—	—	—	—	—
	RR Test Line	—	—	—	—	—	—	—	—
		— indicates where a line will appear on the strip							



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.

Presence of Test lines:

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

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.

The **fourth line** from the top is for CP4EPSPS and it will appear if the sample is positive for CP4EPSPS (RR).

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, Cry2A or CP4EPSPS in individual cotton seed or 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

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

Eurofins 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 Eurofins Amar Immunodiagnostics be liable for incidental, 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.

*Parafilm is a registered trademark of American Can Corporation (now Pechinney Plastic Packaging).

**Bollgard & Roundup Ready are registered trademarks of the Monsanto Company.

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

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