

# Qualitative ELISA for Bt-Cry2Ab

Cat. No: KBA005D

Immunoassay for qualitative screening of Cry2Ab proteins in transgenic crops

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**Introduction:**

*B. thuringiensis* forms crystals of proteinaceous insecticidal  $\delta$ -endotoxins (Cry toxins) which are encoded by **cry** genes. Many genetically engineered crops have been developed with insect tolerance till date by expressing cry genes from *Bacillus thuringiensis*. Bt-modified crops appear to be safe for farmers and consumers.

**Intended Use:**

Bt-Cry2Ab ELISA assay is used as an analytical tool for qualitative laboratory screening of Bt-Cry2Ab proteins in leaves, seedlings and seeds of transgenic crop varieties like cotton. Thus to broaden the range of lepidopteran pests killed and to combat the potential problems with resistance, second generation *Bacillus thuringiensis* plants expressing Cry2Ab toxin alone or in combination with other toxins can be screened.

**Principle:**

The Bt-Cry2Ab Qualitative kit is an Enzyme-Linked Immunosorbent Assay (ELISA).

Plant leaf or seed sample extracts are added to microtiter plate, which is coated with antibodies to Cry2Ab toxin. If Bt-Cry2Ab protein is present in the sample, it binds to the antibody and thus is captured in the wells, which can then be detected by addition of an enzyme conjugate. This immunological reaction results in formation of a sandwich complex of solid phase antibody-toxin-enzyme labeled antibody. The wells are washed to remove any unbound reactants as per the wash procedure (see Assay procedure section mentioned below). The substrate is then reacted. If any enzyme is present the substrate will be hydrolysed signifying the presence of Bt-Cry2Ab protein. This color reaction can be measured spectrophotometrically or observed visually. The color development is proportional to the concentration of Cry2Ab toxin in the sample extract.

**Materials Provided:**

1. Microtiter coated plate (96 wells) – 1 no
2. Enzyme Conjugate, 6 ml – 1 bottle
3. Positive Control – 1 vial
4. Substrate, 11ml – 1 bottle
5. Wash Buffer (20X) – 10 mL
6. Extraction Buffer (20X) – 3 mL
7. Stop Solution, 11 ml – 1 bottle
8. Instruction Manual

**Materials to be provided by the End-User:**

1. Grinding equipment
2. Microplate Reader able to measure absorbance at 450 nm.
3. Adjustable pipettes to measure volumes ranging from 50  $\mu$ l to 25 ml.
4. Deionized (DI) water.
5. Wash bottle or automated microplate washer.
7. Timer.
8. Absorbent paper.

**Handling/Storage:**

1. Reconstitute or dilute only the specific reagents mentioned in the reagent preparation section, when ready to run the assay.
2. Store all kit components at 4<sup>o</sup>C to 8<sup>o</sup>C when not in use and do not expose them to temperatures greater than 37<sup>o</sup>C or less than 2<sup>o</sup>C
3. Do not use kit components after the expiration date.
4. Do not repeatedly freeze/thaw the reagents as loss of activity may result.
5. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
6. ELISA plate pouches contain dessicant. Keep the plates sealed in the pouch with dessicant in the refrigerator when not in use.

**Health Hazard Warnings:**

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.
2. Handle Stop Solution carefully. Obtain medical attention in case of accidental ingestion of kit components.
3. Avoid assay of samples containing Sodium azide as it is hazardous.

**Reagent Preparation (all reagents should be diluted immediately prior to use):**

1. Dilute the 20X **Extraction buffer** to 60 mL to make 1X concentrate.
2. Prepare a sufficient amount of **Wash solution** (1X) by diluting the 20X Wash Buffer (10 mL) to 190 mL of deionized or distilled water. Crystallization of the undiluted wash buffer may occur and can be dissolved by warming.

**Sample preparation:**

The extraction protocols need to be validated by the individual users. Prevent sample-to-sample cross contamination.

*Recommended Protocol –*

- **Leaf samples** – Take green, actively growing leaf samples. Punch 2 to 3 leaf disc samples with the eppendorf in which the extraction is to be carried out and extract it by adding 0.5 to 1mL extraction buffer. Allow the solids to settle and use the extract for the assay
- **Seed sample** – Crush or grind the seed sample into powder and extract it with 0.5 to 1mL of extraction buffer. Allow the solids to settle and use the extract for the assay

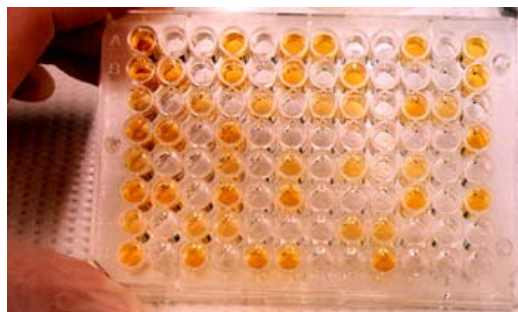
**Procedural Notes:**

1. Read all the instructions thoroughly before performing the test.
2. Allow all reagents to reach room temperature before beginning and reconstitute or dilute the required reagents.
3. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess unreacted reagents is essential.
4. All Standards, Controls and Samples should be assayed atleast in duplicates.

5. If the Substrate has a distinct blue color prior to use it may have been contaminated and use of such substrate can lead to compromisation of the sensitivity of the assay.
6. The assay has been optimized to be used with the protocol mentioned. Any deviation from the same may invalidate the results.

**Assay Procedure:**

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates.
2. Add 50 $\mu$ L of **Extraction buffer** as Blank, 50 $\mu$ L of **Positive Control** and 50 $\mu$ L of **Sample extract** to the respective wells. Cover the microtiter plate with a parafilm.
3. Mix the contents in the wells by moving the plate in rapid circular motion, see to it that the contents do not spill.
4. Incubate at room temperature for 30 minutes or alternatively place on an orbital shaker at about 200rpm for 20 minutes.
5. Add 50  $\mu$ L of **Enzyme Conjugate** to each well and cover the plate with a parafilm and incubate it at room temperature for 60 minutes or use an orbital shaker at 200rpm for 45 minutes.
6. Aspirate and wash plate 4 times with **Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly or alternatively a microtiter plate or strip washer may be used.
7. Add 100 $\mu$ L of **Substrate Solution** into each well of the plate. Cover the plate with a parafilm and incubate for 15 to 30 minutes at ambient temperature or place on an orbital shaker at about 200rpm.
8. Add 100 $\mu$ L of **Stop Solution** to each well within 30 minutes. This will turn the well contents yellow.
9. Measure the optical density of the wells on a plate reader at 450 nm within 30 minutes.



**Interpretation of the Results:**

Compare the OD's of sample extracts with Positive controls and blank wells. Those with absorbance close to blank well are said to be negative i.e. free of Cry2Ab toxin, whereas those with absorbance close to positive control or significantly higher than the blank well are considered positive i.e. transgenic

**Precautions:**

1. Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this set.
2. Substrate is light and heat sensitive hence do not expose it to direct sunlight while pipetting or incubating. Cry2Ab proteins can also be degraded by heat and sunlight.
3. Samples and kit reagents after use should be disposed off observing appropriate regulations.
4. If necessary it is recommended that the results should be confirmed by an alternative method.
5. Do not dilute or adulterate test reagents or use samples not called for in the test procedure.
6. Single leaf and seed samples are by their nature either 100% positive or 100% negative, resulting in a clear delineation of color between negative and positive samples. Low level positive results may be due to insufficient extraction, or can be due to some form of sample cross-contamination or by transfer of particulate matter from leaf or seed extracts into the assay wells. Re-extraction and re-testing of questionable samples is recommended.

**Quality Control:**

It is recommended that for each laboratory assay, appropriate quality control samples in each run to be used to ensure that all reagents and procedures are correct.

**A typical assay setup**

	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	Blank	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
<b>B</b>	P.C	S4	S8	S12	S16	S20	S24	S28	S32	S36	S40	S44
<b>C</b>	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
<b>D</b>	S1	S5	S9	S13	S17	S21	S25	S29	S33	S37	S41	S45
<b>E</b>	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	S46
<b>F</b>	S2	S6	S10	S14	S18	S22	S26	S30	S34	S38	S42	S46
<b>G</b>	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	P.C
<b>H</b>	S3	S7	S11	S15	S19	S23	S27	S31	S35	S39	S43	Blank

\* All controls and samples are run in duplicates

Blank – Blank wells

P.C – Positive control wells

S. – Sample extract wells

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