QUALICHEK™ Soya ELISA Kit

REF : KBFP008

Designed and Developed as per AOAC Official Methods of Analysis Guidelines and European Standards.

Ver 2.1

IVT

Immunoassay for the Quantitative Determination of Soya in food.

IVT	**For In-vitro Testing Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

For In-vitro Testing Only. Not for use in Human or Animal diagnostic or therapeutic procedures. Purchase does not include or carry For Laboratory Use Only the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.

REF **KBFP008** **Σ** **96 tests**

KRISHGEN BioSystems

For US/Europe Customers: toll free +1(888)-970-0827|tel +1(562)-568-5005
For Asia/India Customers: +91(22)-49198700
Email: sales@krishgen.com | http://www.krishgen.com

Introduction:

Soy (also called Glycine max) and more commonly known as soybean is a member of the legumes that are native to East Asia. These beans are rich in dietary minerals, B vitamins and phytic acid. Soy vegetable oil is often used in many industrial and food applications. The fraction of proteins in soy beans is relatively high (approx. 40%). It is these proteins which lead to allergic reactions (for example: Glycinin, Gly m1, Gly m4 and Kunitz-TrypsinInhibitor). For an individual that is allergic to soy any hidden soy allergens that are present in food can result in critical problems, even a small amount can lead to allergic reactions and in severe cases cause anaphylactic shock.

Intended Use:

QUALICHEK™ Soya ELISA kit is sandwich enzyme immunoassays for the quantitative determination of Soya protein in processed or unprocessed food. on the base of STI and is particularly capable of the quantification of soy residues in cookies, cereals, ice cream, chocolate, instant soups and sausage.

Principle:

QUALICHEK™ Soya ELISA kit is based on the principle of the enzyme linked immunosorbent assay for the detection of Soya Beta-conglycinin protein in the sample. The antibodies against Soya Beta-conglycinin protein are pre-coated on the micro-well strips. The Soya Beta-conglycinin protein in the sample and the antibodies pre-coated on the micro-well strips will form immune complex. Unbound materials are removed by washing. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The colour development is stopped by the addition of a stop solution. The intensity of the color is measured photometrically at 450 nm. The concentration of Soya Beta-conglycinin protein is directly proportional to the color intensity of the test sample.

Materials Provided:

1. Anti-Soy monoclonal antibody coated microtitre well plate, (8x12 strips) - 1 no
2. Soy (STI) Standards (0, 40, 100, 400 and 1000 ppb) - 2 ml each
3. Anti-STI (Soy Trypsin Inhibitor) HRP:Conjugate - 15 ml
4. (10X) Reagent A - 2 x 120 ml
5. TMB Substrate - 15 ml
6. Stop Solution - 15 ml
7. (10X) Wash Buffer - 60 ml
8. Instruction Manual

Materials Required but not Provided:

1. Distilled Water (or Deionized (DI) Water)
2. Micropipettes and disposable tips ranging from 50 to 1000 ul
3. Graduated cylinders
4. Polypropylene centrifuge tubes (50 ml size)
5. Polypropylene micro tubes (1 - 2 ml size)
6. Homogenizer/blender for sample preparation (if necessary)
7. Centrifuge
8. Water Bath
9. Vortex mixer
10. Aspirator for washing procedure, or optional microplate washer
11. Microplate reader with a 450 nm filter

Handling/Storage:

1. Store main kit components at recommend temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin.

Reagent Preparation before Sample Preparation:

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

1. Label any aliquots made with the kit Lot No and Expiration date and store it at appropriate conditions mentioned.
2. Bring all reagents to Room temperature before use.
3. **To prepare Sample Extraction Solution**; dilute **120 ml Reagent A** with **1080 ml DI/Distilled Water**.
   Note:
   a. Reagent A may produce crystals after refrigerated storage. These crystals must be re-dissolved completely in a water bath at 30-37°C prior to use. The fully re-dissolved Reagent A can be stored at 20-30°C.
   b. Sample Extraction Solution can be stored at 4°C for 7 days. If Sample Extraction Solution forms a precipitate / crystals after refrigerated storage, then the solution must be warmed in a water bath at 20-30°C to re-dissolve the precipitate / crystals prior to use.
4. **To make a (1X) Wash Buffer**; dilute **60 ml of (10X) Wash Buffer** with **540 ml DI/Distilled Water**.

Sample Preparation:

The following sample preparation should be applied for all kinds of solid samples:

1. To maximize homogeneity and representativeness of the sample collection, a minimum of 5 gm sample should be pulverized finely in a mortar, impact mill etc.
2. 1 gm of the homogenized mixture is suspended in 20 ml of Sample Extraction Solution. Afterwards the suspension is incubated for 15 mins in a preheated water bath at 60ºC. To ensure good homogeneity, the samples should be shaken well every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 rpm. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. If the results of a sample are out of the assay range, further dilution with the Sample Extraction Solution is necessary. The additional dilution has to be considered when calculating the concentration of the sample during Results Interpretation.

The following sample preparation should be applied for all kinds of liquid samples:

1. To maximize homogeneity and representativeness of the sample collection, a minimum of 5 ml sample should be collected.
2. 1 ml of the sample is diluted in 9.5 ml of Sample Extraction Buffer. Afterwards the suspension is incubated for 15 mins in a preheated water bath at 60ºC. To ensure good homogeneity, the samples should be shaken well every two minutes.
3. The samples are centrifuged for 10 minutes at 2000 rpm. If it is not possible to separate the supernatant from the precipitate completely, the suspension should be filtrated if necessary.
4. If the results of a sample are out of the assay range, further dilution with the Sample Extraction Solution is necessary. The additional dilution has to be considered when calculating the concentration of the sample during Results Interpretation.

Procedural Notes:

1. In order to achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess un-reacted reagents is essential.
2. Avoid assay of Samples containing sodium azide (NaN_3), as it could destroy the HRP activity resulting in under-estimation of the amount of Soy.
3. It is recommended that the Standards and Samples be assayed in duplicates.
4. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are same for each well.
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 plates should be read within 30 minutes after adding the Stop Solution.
7. Make a work list in order to identify the location of Standards and Samples.

Assay Procedure:

1. Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. All steps must be performed at room temperature.

2. Add **100 ul** of **Standards or prepared Samples** into respective wells.

3. Cover the plate with plate sealer, and incubate for 20 mins at Room Temperature.

4. Aspirate and wash plate 3 times with Wash Buffer (1X) 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.

5. Add **100 ul** of **Anti-STI (Soy Trypsin Inhibitor):HRP Conjugate** into each well. Note the solution is colored red for easy to use pipetting.

6. Cover the plate with plate sealer, and incubate for 20 mins at Room Temperature.

7. Aspirate and wash plate 3 times with Wash Buffer (1X) 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.

8. Add **100 ul** of **TMB Substrate** into each well and incubate in dark for 20 mins at Room Temperature.

9. Add **100 ul** of **Stop Solution** into each well.

10. Read the plate at 450 nm with a microplate reader.
    Note: The absorbance must be measured within 30 minutes after stopping the enzyme reaction.

Interpretation of Results:

Determine the Mean Absorbance for each set of duplicate Standards and Samples. Using standard graph paper, plot the average value (absorbance 450nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown Soy (STI) protein concentrations, find the unknown's Mean Absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the concentration. If samples were diluted, multiply by the appropriate dilution factor.

Software which is able to generate a polynomial regression (2nd order) or a cubic spline curve-fit is best recommended for automated results. If a data processing software is required, please contact us at sales@krishgen.com for the same.

Multiply the results with the appropriate conversion factor indicated below based on the sample type.
Note: If the samples were further diluted using the Sample Extraction Solution to bring the results within the assay range, multiply by the dilution factor.

The following conversion factors have been determined by means of in-house validation:

Soy Flour, unroasted	conversion factor = 42
Soy Flour, roasted	conversion factor = 470
Soy Protein Isolate (90%)	conversion factor = 864
Soy Milk	conversion factor = 2500
Tofu	conversion factor = 50000

Note:
It is recommended to repeat the assay at a different dilution factor in the following cases:
- If the sample absorbance value is below the first standard.

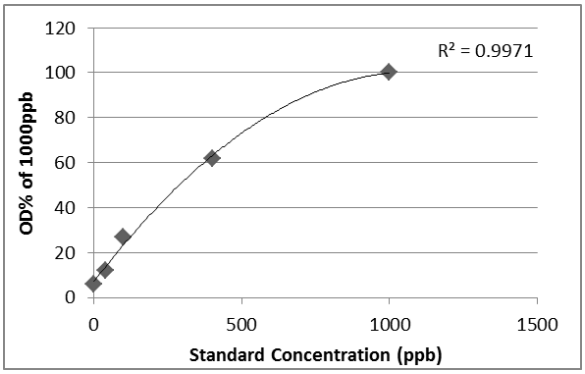
The detectable protein content in a sample, in ppm, can be estimated using the following formula:
Soy (STI) Protein content 1 ug/ml = 1000 ppb = 1 ppm

Conversions:
1 mg/l = 1 ppm = 1,000 ppb
1 mg/ml = 1,000 ppm = 106 ppb
1% = 1 gm/dl = 10,000 ppm = 107 ppb

Typical Standard Curve

Soy (ppb)	Mean Abs
0	0.11
40	0.23
100	0.49
400	1.12
1000	1.8

Typical Graph



Abs = absorbance

Cautionary Note: In the case of processed foods, the detection efficiency or sensitivity of the assay may decrease, due to denaturation and insolubilization of proteins. Therefore, food samples that give a negative result may still contain allergic ingredient which is either unreactive or present at concentrations below the limit of detection. It should not be assumed that such foods are allergic ingredient free.

Limitation of the Procedure:

This ELISA test is designed for quantitative detection of Soy in samples only.

Performance Characteristics:

Sensitivity:
Limit of Detection (LOD):
16 ppb (16 ug detectable protein*/gm food)
Limit of Quantification (LOQ):
40 ppb (40 ug detectable protein*/gm food)
Due to the variety of sample matrices and their influence on the absorbances, results less than the LOQ should be treated as negative.

Specificity:
Soy beans have over 39% protein fractions including the allergenic ones Gly m1, Glycinin, Kunitz-Trypsin-Inhibitor and Gly m4. The kit uses antibodies specific for STI (soy trypsin inhibitor). Cross-reactivity was observed for for sesame: 0.0002 %. No cross reactivity was observed for Rice, Corn, Almond, Gelatin, Barley, Pea, Cow's Milk, Egg, Pork, Rye, Chickpea, Peanut, Cocoa, Beef, Oats, Bean, Hazelnut, Sugar, Chicken.

Assay Range:
0 - 1000 ppb

Precision:
Intra-Assay precision: < 15%
Inter-Assay precision: < 18%

Spike Recovery:
The following samples of known concentrations of Gliadin was spiked in the sample extraction solution and mean recovery calculated -

Cookies	106 %
Cereals	100 %
Ice cream	77 %
Chocolate	77 %
Instant soup	90 %
Sausage	96 %

Safety Precautions:

- **This kit is For In-vitro Testing Only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

Advice on Handling the Test:

Reliability of the test results
In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, NABL, etc.). In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions. It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.

Complaints
In case of complaints please submit a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout.

Disposal
Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.

Interference
Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation. All reagents of this test kit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.

LIMITED WARRANTY

Krishgen Biosystems does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Biosystems, or against damages resulting from such non-Krishgen Biosystems made products or components. Krishgen Biosystems passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Biosystems.

THIS WARRANTY IS EXCLUSIVE. The sole and exclusive obligation of Krishgen Biosystems shall be to repair or replace the defective product in the manner and for the period provided above. Krishgen Biosystems shall not have any other obligation with respect to the products or any part thereof, whether based on contract, tort, and strict liability or otherwise.

Under no circumstances, whether based on this Limited Warranty or otherwise, shall Krishgen Biosystems be liable for incidental, special, or consequential damages.

This Limited Warranty states the entire obligation of Krishgen Biosystems with respect to the product. If any part of this Limited Warranty is determined to be void or illegal, the remainder shall remain in full force and effect.

Krishgen Biosystems, 2022

THANK YOU FOR USING A KRISHGEN PRODUCT!

PROTOCOL SUMMARY

Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells. It is strongly recommended that all Standards and Samples be run in duplicates or triplicates. All steps must be performed at room temperature.

↓

Add 100 ul of Standards or prepared Samples into respective wells.

↓

Cover the plate with plate sealer, and incubate for 20 mins at Room Temperature.

↓

Aspirate and wash plate 3 times with Wash Buffer (1X) 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.

↓

Add 100 ul of Anti-Gliadin:HRP Conjugate into each well.

↓

Cover the plate with plate sealer, and incubate for 20 mins at Room Temperature.

↓

Aspirate and wash plate 3 times with Wash Buffer (1X) 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.

↓

Add 100 ul of TMB Substrate into each well and incubate in dark for 20 mins at Room Temperature.

↓

Add 100 ul of Stop Solution into each well.

↓

Read the plate at 450 nm with a microplate reader.