AgraQuant®
Toasted Meal Plate

TOASTED MEAL – SEED & LEAF

Order Number 7099999

Product Description

The AgraQuant® Toasted Meal Plate - GMO✓ is designed to detect the CP4 EPSPS protein expressed in Roundup Ready® soybeans. The kit has been developed to identify this protein in Soya Processed Food Fractions such as toasted meals, concentrates and isolates. For quantitative analysis, additional standards of protein isolates can be purchased separately (7100004).

Contents of Kit	Quantity
AgraQuant® Toasted Meal Plate - GMO✓ ELISA microtiter plate wells	12 strips of
Soya Extraction Buffer	500mL
RUR Conjugate 1	15mL
RUR Conjugate 2	15mL
Color Reagent	20mL
Stop Solution	20mL
10X Wash Buffer Concentrate 100mL	
Plate Sealers	3

Materials Required but not Supplied:

GMOChek Soya Standards at 0, 0.3%, 1.25%, and 2.5%, 3grams each
(a) Soya full-fat flour (FFF) Standards (7100001)
(b) Soya Defatted Flour (DF) Standards (7100002)
(c) Soya Protein Isolate (PI) Standards (7100004)
12 x 75 mm glass test tubes
15mL polypropylene conical centrifuge tubes (COSST5043)
40mL polypropylene conical centrifuge tubes (LABSP1239)
Transfer pipets (COOLS2010)
Weigh boats or Equivalent (LABSP1028)
Spatulas (EQOLE1250)
Laboratory Tape
Wash bottle for manual plate washing (COKAD1150)
Balance capable of 0.005 gram measurement.
Precision pipettes: 20 µL - 200 µL (EQOLE1183), 200 µL - 1000 µL (EQOLE1130)
Pipet tips: 20 µL - 200 µL (COOLS1139), 200 µL - 1000 µL (LABSP1272)
Vortex mixer (EQOLE1335)
Microtiter plate reader capable of reading absorbance at 450 nm (preferably with subtraction of 650 nm absorbance capability, i.e. StatFax 303+) (EQOLE1409)

Materials Recommended but not Provided

Multi-channel pipette (EQOLE1163)
Reagent reservoirs for muli-channel pipetting (COOLS1101)
Automated plate washer (EQOLE1477)
Test tube rack for 15mL centrifuge tubes (EQOLE1220)

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Storage and Preparation of Reagents

The AgraQuant® Toasted Meal Plate - GMO✓ ELISA plate kit should be stored at 2 – 8ºC. Do not freeze. Remove reagents from refrigerated storage at least 30 minutes prior to use. Check the desiccant packet in the microplate foil pouch to be sure the indicator pellets in the packet are blue in color. A pink color indicates the desiccant is no longer active. If pink, contact Romer® Labs, Inc. Technical Service.

Test Preparation

Note: Allow all reagents to warm to room temperature before using.

Preparation of Soya Coated Strips for automated or manual plate washing:
If using an automated plate washer: Remove the (8) RUR Coated Strips and Strip Holder from the foil bag. If less than a full plate of samples will be run, replace the required number of RUR Coated Strips with Uncoated Strips (not included in the kit). Place unused RUR Coated Strips back into the foil bag and seal the reclosable end. Using the Semi-Quantitative Procedure, four (4) wells of one strip are required for the Controls (Figure 1). Using the Quantitative Procedure 8 wells of one strip are required to run the four standards in duplicate (Figure 2). For additional runs, a new set of Standards must be run. Always seal the foil bag, each time, after removing the RUR Coated Strips.

If using manual washing: Remove the (8) RUR Coated Strips and Strip Holder from the foil bag. Arrange the required number of RUR Coated Strips into the strip holder. Place the unused RUR Coated Strips immediately back into the foil bag and seal the ziploc end. Tape the edges of each strip to the strip holder to prevent strips from accidentally falling out during the Wash Step. As illustrated in Figure 1, four (4) wells of one strip are required for the Controls. Using the Quantitative Procedure, 8 wells of one strip are required to run the four standards in duplicate (Figure 2). For additional runs, a new set of Standards must be run. Always seal the foil bag, each time, after removing the RUR Coated Strips.

Preparation of Wash Buffer

1. Allow the 10X Buffer Concentrate to come to room temperature.
2. Dilute the 10X Buffer Concentrate in De-ionized water to prepare the Working Assay Buffer. *Example: 50 mL 10X Buffer Concentrate into 450 mL deionized water.*
3. Add the required volume to the Automatic Plate Washer or Wash Bottle (Fisher Scientific 18oz -500 mL Nalgene Wash Bottle #03-409-10E, or equivalent).

Sample Preparation for Qualitative Analysis

a) For quantitative analysis, additional standards of protein isolates can be purchased separately (Part # 7100004). Standards are at 0%, 0.3%, 1.25% and 2.5% RUR soya. Each of these standards can be used for quantitative determination if the following table is used for standard weight and extraction volume.
For example, if the Protein Isolate (PI) Standards are used to quantitate Toasted Meal samples, 0.10 grams of each standard are extracted in 7 mL of Extraction Buffer and 1.0 gram of each toasted meal sample are extracted in 10 mL of Extraction Buffer. The absorbances obtained with the Toasted Meal samples are then compared to the PI standards for quantitative interpretation.

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b) Weigh out the appropriate amount of sample or standard into the appropriate container. Weigh ±0.005g. *Note: Clean saptula between samples.*

Soya Fraction	Weight of Fraction (g)	Volume of Extraction Buffer (mL)	Vortex Time (min.)
Toasted Meals	1.0	10	1
Protein Isolates	0.10	7	3
Protein Concentrates (Acid Leached)	0.10	10	3
Protein Concentrates (Alcohol Washed)	1.0	6	3
Full-Fat Flour	0.1	16	1
Defatted Flour	0.1	32	1
Ground Soybeans (passed through 40 US Mesh)	0.1	13	1

c) Pipette the appropriate amount of Soya Extraction Buffer into tube containing the sample and vortex for at least one minute (3 minutes for Protein Isolates and Concentrates).
d) Allow the extracted samples to settle for 5 minutes after the last extraction. Immediately begin sample addition in the order the samples were extracted.

NOTE: *Run soya extracts immediately after extraction. Soya extracts and diluted extracts CANNOT be used for subsequent runs.*

Assay Procedure

Sample Addition:

Add 100 µL of each extract in duplicate from step (d) of the Extraction Procedure for the Quantitative assay to the appropriate wells (Refer to Figure 1). Cover plate with supplied plate sealer to prevent contamination and evaporation.

2. ***Sample Incubation***:

Incubate assay strips at room temperature for 1 hour.

3. ***Wash Cycle:***

a) Wash 4 times with Wash Buffer (300 µL /well). If an automated plate washer is not available, manual washing can be performed as follows (be sure the strips are taped to the holder):
b) Invert the taped strip holder and discard the contents into a sink or suitable waste container. Tap the inverted strip holder onto a stack of paper towels to remove residual sample.
c) Add Wash Buffer to each well of the RUR Coated Strips with the Wash Buffer bottle (Fisher Scientific 18oz -500 mL Nalgene Wash Bottle #03-409-10E , or equivalent). Fill each well with an overflow volume of Wash Buffer.

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d) Invert the Strip holder and discard the contents into a sink or suitable waste container. Tap the inverted strip holder onto a stack of paper towels to remove residual wash buffer.

e) Repeat steps (c) and (d) above three more times.

NOTE: <u>Do not let wells dry out</u>, as it may affect assay performance.

4. ***Addition of RUR Conjugate 1:***

   Add 100 µL of the RUR Conjugate 1 to each well of RUR Coated Strips. Cover plate with supplied plate sealer to prevent evaporation.

5. ***RUR Conjugate 1 Incubation:***

   Incubate assay strips at room temperature for 30 minutes.

6. ***Wash Cycle:***

   Wash 4 times with Wash Buffer as described in step 3 above.

7. ***Addition of RUR Conjugate 2***

   Add 100 µL of the RUR Conjugate 2 to each well of RUR Coated Strips. Cover plate with supplied plate sealers to prevent evaporation.

8. ***RUR Conjugate 2 Incubation:***

   Incubate assay strips at room temperature for 30 minutes.

9. ***Wash Cycle:***

   Wash 4 times with Wash Buffer as described in step 3 above.

10. ***Color Development:***

    (a) Add 100 µL of Color Solution to each well of the RUR Coated Strips and incubate for 30 minutes at room temperature.

    (b) After the 30 minute incubation, stop color development by adding 100 µL of Stop Solution to each well of RUR Coated Strips in the same sequence that the Color Solution was added.

    (c) Read the absorbance of the developed color at 450 nm using a microtiter plate reader.

<u>Raw Data Log</u>

If an automated plate reader print out is not available, record the raw data on the attached Data Log, Figure 2.

<u>Accept / Reject Criteria</u>

The following Accept/Reject Criteria is **recommended**:

A <u>run</u> is considered acceptable only if the following criteria are met:

(a) The mean of duplicate determinations of the 0% Negative Standard OD at 450 nm is ≤0.15

(b) The mean of duplicate determinations of the 2.5% Standard OD at 450 nm is ≥ 0.8 and ≤2.2

(c) The %CV of duplicates for the 0.30%, 1.25% and 2.5% Standards must be ≤15%.

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(d) The correlation coefficient (r2) generated from the quadratic analysis of the Standards
must be greater than 0.98. The correlation coefficient (r) generated from the linear regression analysis of the Standards must be greater than 0.96.

Data Interpretation

Quantitative Analysis

Average the absorbance readings for the duplicate standards and unknowns.

Plot a standard curve of the mean absorbance (OD) vs. %GMO of the Standards using a quadratic fit (ax^2+bx+c). Perform a regression analysis of the standards, including the 0% standard to obtain a best-fit line. An EXCEL spreadsheet can be provided for the regression analysis by contacting the SDI Technical Service Department.

Interpolate the unknown sample concentrations from the standard curve using the regression equation. Ensure the accept/reject results are within accepted quality control criteria prior to reporting sample results.

Precautions

- Store all test components refrigerated (2-8°C). Storage at ambient temperature on the day of use is acceptable.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Allow reagents to reach ambient temperature (18-27°C or 64-81°F) before beginning the test. This typically requires at least 1 hour to warm from recommended storage conditions with the contents out of the package.
- Do not use components after their expiration date.
- Do not expose Color Reagent to direct sunlight.
- Do not mix reagents from different test kit lots.
- Do not dilute or adulterate test reagents or use samples not called for in the test procedure. This may give inaccurate results.

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