



User's Manual

Capsaicin ELISA Kit



DEIASL105



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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

The Capsaicin ELISA Kit is an immunological laboratory test for the quantitation of capsaicin in raw peppers and salsa.

Principles of Testing

Calibrators and the Sample Extract(s) are pipetted into the mixing wells followed by Capsaicin HRP Enzyme Conjugate. The reagents are mixed and transferred to the test wells to initiate the reaction. During an incubation, Capsaicin in the calibrator/sample and Capsaicin HRP Enzyme Conjugate compete for binding to the polyclonal Capsaicin antibody immobilized on the test wells surface. Following the incubation, the wells are washed to remove any unbound Capsaicin and Capsaicin HRP Enzyme Conjugate. After washing, a colorless substrate is added to the wells and any bound enzyme conjugate will convert the substrate to a blue color. Following an incubation, the reaction is stopped with the addition of Stop Solution and the amount of color in each well is measured. The color of the unknown sample is compared to the color of the calibrators and the Capsaicin concentration of the sample is derived.

Reagents And Materials Provided

1. 1 Unit Plate containing 12 strips of 8 test wells each that are vacuum sealed in an aluminized pouch with a desiccant.
2. 1 Unit Plate containing 12 strips of 8 mixing wells each that are packaged in a zip-loc bag.
3. 4×2 mL Vials of Capsaicin(Natural Mixture) Calibrators(0, 0.1, 0.5 and 2 ppm).
4. 1×12 mL Bottle of Capsaicin HRP Enzyme Conjugate
5. 1×14 mL Bottle of Substrate.
6. 1×14 mL Bottle of Stop Solution.

Materials Required But Not Supplied

1. Pipette(s) with disposable tips capable of dispensing the required volume(s).
2. Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s).
3. Laboratory quality distilled or deionized water.
4. Reagents and materials for sample preparation.
5. Personal protective equipment.
6. Paper towels or equivalent absorbent material.
7. Wash bottle (optional).
8. Timer.
9. Microtiter plate or strip reader capable of reading at 450 nm.

Specimen Collection And Preparation

Raw Pepper and Salsa:(Dilution Factor: 50)

1. Puree a representative sample in a blender for 2 minutes
2. Weigh 5 g of the pureed sample into a 50 mL centrifuge tube
3. Measure 25 mL of 100% methanol and add to the centrifuge tube.
4. Homogenize the mixture using a Polytron for 3 minutes at medium speed.
5. Centrifuge for 10 minutes at 15,000 x g.
6. Dilute the supernatant 1:10 in laboratory quality distilled or deionized water and use in the assay.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of mixing wells and test wells into a holder. Be sure to reseal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense 100 uL of Calibrators and Sample Extract(s) into the appropriate mixing well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense 100 uL of Enzyme Conjugate into each mixing well.
5. Mix the contents of each well by gently pipetting up and down with a multichannel pipette and transfer 100 ul of the mixture to the test wells.
6. Incubate the test wells for 10 minutes at room temperature. Discard the mixing wells.
7. Decant the contents of the wells into an appropriate waste container. Fill the wells to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step four times for a total of five washes. Following the last wash, tap the inverted wells onto absorbent paper to remove excess wash solution.
8. Dispense 100 ul of Substrate into each well.
9. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for 10 minutes at room temperature.
10. Dispense 100 uL of Stop Solution into each well in the same order of addition as the Substrate.
11. Gently shake the wells for 30 seconds using a back-and-forth motion.
12. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each well at 450 nm using a plate or strip reader within 10 minutes of stopping the assay. If the reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
13. Dispose of used test wells in an appropriate waste container.

Quality Control

The correlation coefficient (R^2) of the calibration curve, analyzed using a 4-parameter logistic regression, must be ≥ 0.99 .

The average absorbance of the zero calibrator replicates must be ≥ 1.0

The average absorbance of calibrator replicates must have a coefficient of variation (%CV)<15%.

The average absorbance of sample replicates must have a coefficient of variation (%CV) <20%.

Calculation

It is preferred for quantitative results to be determined using commercially available software for ELISA evaluation using a 4-parameter curve fit. Alternatively, a semi-log curve fit can be used if 4-parameter software is not available. A spreadsheet that will perform the curve fit and sample concentration calculations is available upon request.

To ensure the validity of the results, please adhere to the following:

1. Ensure QC criteria are met.
2. The concentration of Capsaicin in a sample is determined by comparing the average sample absorbance to the standard curve. This value must then be multiplied by the dilution factor used.
3. In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 10% methanol to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.

Specificity

The Capsaicin RS Plate Kit is specific for Capsaicin with reactivity to a limited number of closely related compounds. The following table shows the relative values for 50% B₀ and the percent cross-reactivity versus Capsaicin(Natural Mixture). All concentrations are in ppm.

Compound	50% B ₀	%Cross-Reactivity
Capsaicin(Natural Mixture)*	0.625	100
Capsaicin (Pure)	0.599	104
Dihydrocapsaicin	0.639	98

*Contains approximately 65% Capsaicin and 35% Dihydrocapsaicin.

Precautions

1. Read the product brochure in its entirety prior to use.
2. The kit, in its original packaging, can be used until the end of the month indicated on the box label.
3. Do not use reagents after expiration date.
4. Store all kit components at 2°C to 8°C(36°F to 46°F) when not in use.
5. Reagents should be brought to room temperature, 20°C to 28°C(68°F to 82°F), prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
6. Do not freeze kit components or expose them to temperatures greater than 37°C (99°F)
7. Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
8. Precise transfer of samples and reagents by using a calibrated pipette that is capable of dispensing the required volume is critical to obtain proper assay results.
9. If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.

10. All procedural steps should be completed without interruption. Ensure all reagents materials and equipment are ready at the appropriate time.
11. Each reagent is optimized for use in the Capsaicin Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Capsaicin Plate Kits with different lot numbers.
12. Do not reuse test wells.
13. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
14. Damage to or obstruction of the optical surface may cause unsatisfactory results.

