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User's Manual

Capsaicin ELISA Kit

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Σ **96T**

RUO

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.

General Description

Capsaicin is most often used as a topical analgesic and exists in many formulations of cream, liquid, and patch preparations of various strengths; however, it may also be found in some dietary supplements. Capsaicin is a naturally-occurring botanical irritant in chili peppers, synthetically derived for pharmaceutical formulations. The most recent capsaicin FDA approval was Qutenza, an 8% capsaicin patch dermal-delivery system, indicated for neuropathic pain associated with post-herpetic neuralgia.

Principles of Testing

The Capsaicin ELISA Kit uses a polyclonal antibody that binds both capsaicin and a capsaicin-enzyme conjugate. Capsaicin in the sample competes with the capsaicin-enzyme conjugate for a limited number of antibody binding sites. Antibodies, which bind capsaicin, are immobilized to the inside of the test wells. In the assay procedure you will:

• Add a mixture of a sample containing capsaicin and capsaicin-enzyme conjugate to a test well. The conjugate competes with any capsaicin in the sample for the same antibody binding sites.

• Wash away any unbound molecules, after you incubate this mixture for 10 minutes.

• Add clear substrate solution to each well. In the presence of bound capsaicin-enzyme conjugate, the substrate is converted to a blue compound. One enzyme molecule can convert many substrate molecules.

Since the same number of antibody binding sites are available in every well, and each well receives the same number of capsaicin-enzyme conjugate molecules, a sample containing a low concentration of capsaicin allows the antibody to bind many capsaicin-enzyme conjugate molecules. The result is a dark blue solution. Conversely, a high concentration of capsaicin allows fewer capsaicin-enzyme conjugate molecules to be bound by the antibodies, resulting in a lighter blue solution.

NOTE: Color is inversely proportional to capsaicin concentration.

Darker color = Lower concentration

Lighter color = Higher concentration

Reagents And Materials Provided

1	Plate containing 12 strips of 8 wells coated with rabbit anti-capsaicin antibodies
1	Bag containing 12 strips of 8 mixing wells
1	Vial Negative Control (0.0 ppm capsaicin)
3	Vials Capsaicin (natural mixture) Calibrator (0.1 ppm, 0.5 ppm, and 2.0 ppm)
1	Vial Capsaicin-HRP Enzyme Conjugate

1 Vial Substrate

1 Vial Stop Solution (Caution! Contains 1N HCl. Handle with care.)

Materials Required But Not Supplied

• Laboratory quality distilled or deionized water.

• Pipette with disposable tips capable of dispensing 100 µL.

• Multi-channel pipette with disposable tips; 8 channels capable of dispensing 100 µL.

• Paper towels or an equivalent absorbent material.

• Microwell plate or strip reader with 450 nm filter.

• Wash bottle.

• Timer.

*Additional materials may be required for sample preparation. See Sample Preparation Protocol.

Storage

The kit in its original packaging can be used until the end of the month indicated on the box label when stored at 2 – 8°C.

Specimen Collection And Preparation

1. Puree a representative sample of salsa or raw pepper in a blender for 2 minutes to ensure a homogeneous sample.
2. Weigh 5 grams of the pureed sample into a 50 mL conical centrifuge tube and add 25 mL methanol.
3. Homogenize the mixture using a Polytron for 3 minutes at medium speed.
4. Centrifuge for 10 minutes at 15,000 x g. Remove and save supernatant.
5. Dilute supernatant 1:10 in laboratory grade water. If further dilutions are required to bring the sample concentration within the range of the curve, serially dilute in 10% methanol/water.

Assay Procedure

(Note: Running calibrators and samples in duplicate will improve assay precision and accuracy.)

1. Allow all kit reagents and samples to warm to room temperature.
2. Remove the required number of red labeled mixing wells from the plastic bag. Remove an equal number of antibody coated strips from the re-sealable foil bag. Be sure to re-seal the bag with the desiccant to limit moisture exposure.
3. Pipette 100 µL of Calibrators or Samples into the appropriate mixing wells. Be sure to use a clean pipette tip for each solution.
4. Add 100 µL of Enzyme Conjugate to each mixing well.
5. Mix the contents of each well gently by pipetting up and down 4-5 times with a multichannel pipette.

Transfer 100 µl of the mixture to the antibody coated reaction wells.

6. Shake the plate gently for 30 seconds using a back and forth motion to mix the contents. Alternatively, the plate may be incubated on a rotator for continuous mixing during incubation. Cover the wells with tape or Parafilm.
7. Incubate for 10 minutes at room temperature. Discard the used mixing wells.
8. Remove the covering and decant the contents of the wells into an appropriate waste container. Fill the wells completely with cool running tap water and then decant. Repeat this wash step four times for a total of five washes.
9. Following the last wash step, tap the inverted wells onto absorbent paper to remove the last of the water.
10. Dispense 100 µL of Substrate to each well.
11. Cover the wells and incubate for 10 minutes at room temperature.
12. Dispense 100 µL of Stop Solution to each well in the same order of addition as the Substrate. WARNING!: Stop Solution is 1N HCl. Handle with care.
13. Read the plate on a microtiter plate reader at 450 nm. If the plate reader has dual wavelength capability, read at 450 nm minus 605 or 650 nm.
14. If the microtiter plate reader has data reduction capabilities, use either a semi-log linear or 4-parameter curve fit. If manual data reduction is required, proceed as in the calculate results section.

Calculation

1. After all of the wells have been read, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:

%Bo = (average OD of calibrator, control or sample x 100) ÷ average OD of negative control

2. Graph the %Bo of each calibrator on the Y (linear) axis against its concentration on the X (log) axis using semi-log graph paper. Draw the best-fit line through the calibrator points.
3. Determine the capsaicin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph and multiply by the appropriate dilution factor.
4. Calculation of sample concentration is only valid if the %Bo of the sample falls within the range of the %Bo's set by the calibrators. If the sample falls outside of that range, the results must be reported as less than the lowest calibrator value or greater than the highest calibrator value.

Typical Standard Curve

Well Contents	OD	Avg. ± SD**	%RSD	%Bo	Capsaicin (ppm)
Negative Control	1.574 1.533	1.553 ± 0.029	1.86	100	N/A
0.1 ppm Calibrator	1.250 1.281	1.265 ± 0.022	1.73	81	N/A
0.5 ppm Calibrator	0.764 0.739	0.751 ± 0.018	2.35	48	N/A
2.0 ppm Calibrator	0.354 0.335	0.344 ± 0.013	3.91	22	N/A
Sample	0.858 0.877	0.868 ± 0.013	1.55	56	0.35

Actual values may vary; this data is for example purposes only.

* standard deviation

** %Bo equals average sample absorbance divided by average negative control absorbance times 100%

Specificity

The Capsaicin ELISA Kit is specific for capsaicin with reactivity to a limited number of closely related compounds. The following table shows the relative values for 50% Bo and the percent cross-reactivity (%CR) versus capsaicin (natural). All concentrations are in parts per million (ppm).

Compound	50% B_o	%CR
Capsaicin (natural mixture)*	0.625	100
Capsaicin (pure)	0.599	104
Dihydrocapsaicin	0.639	98

*Contains ~ 65 % capsaicin and 35 % dihydrocapsaicin

Precautions

1. Store all kit components at 4°C to 8°C (39°F to 46°F) when not in use.
2. Each reagent is optimized for use in the Capsaicin ELISA Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Capsaicin ELISA Kits with different lot numbers.
3. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
4. Do not use reagents after expiration date.
5. Do not freeze the plate kit components or expose them to temperatures greater than 37°C (99°F).
6. Reagents should be brought to room temperature, 20 – 28°C (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
7. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.
8. Transfer of samples and reagents by pipette requires constant monitoring of technique. Pipetting errors are the major source of error in immunoassay methodology.
9. If running more than two strips at once, the use of a multichannel pipette is recommended.
10. Use approved methodologies to confirm any positive results.