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User's Manual

High Sensitivity Capsaicin ELISA Kit

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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 High Sensitivity Capsaicin ELISA Kit is an immunological laboratory test for the quantitation of Capsaicin in Dehydrated Peppers and Oleoresins.

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 Capsaicinenzyme 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 antiCapsaicin antibodies

1 vial of Negative Control (0.0 ppb Capsaicin)

1 vial each of 1 ppb, 10 ppb, and 100 ppb Capsaicin (natural mixture) Calibrator

1 vial of Capsaicin-HRP Enzyme Conjugate

1 vial of 10X phosphate buffered saline (PBS)
1 vial of Substrate
1 vial of Stop Solution
1 Instructional Booklet

Materials Required But Not Supplied

• Microtiter plate reader
• Tape or Parafilm®
• Watch or timer
• Clean running water or a wash bottle containing tap or deionized water.
• Orbital shaker (optional)
• Miscellaneous glassware for dilutions

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

For Dehydrated Pepper samples and Oleoresins:

1. Weigh 0.1 g dehydrated pepper or oil sample into a small vial.
2. Add 10 ml 100 % methanol. For extraction of dehydrated pepper samples incubate for 1 hour at room temperature with vortexing every 10-15 min. For extraction of oleoresins incubate 30 min at room temperature with vortexing every 5 to 10 min. (sample is diluted 1:100 fold at this stage)
3. Dilute extracts as required using a positive displacement pipette. (ex. Dilute low heat variety samples 1:10 and high heat variety samples 1:1000 in methanol)
4. Final extract preparation. Add 0.1 ml Methanolic extract to 0.9 ml of phosphate buffered saline (PBS) to reduce organic solvent interference.

Assay Procedure

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

1. Warm all kit reagents and samples to room temperature.
2. Dilute 25 mL vial of 10X PBS to 250mL with deionized water.
3. Prepare calibrators by adding 0.1 ml of provided calibrator stocks to 0.9 ml of phosphate buffered saline (PBS) to reduce organic solvent interference. The use of a positive displacement pipette is recommended.
4. Remove the required number of antibody coated strips from the foil bag. Be sure to re-seal the bag with the desiccant to limit exposure of the strips to moisture.
5. Pipet 100 µL of calibrators and samples into the appropriate wells. Be sure to use a clean pipette tip for

each solution to avoid cross contamination.

6. Add 100 µL of Enzyme Conjugate to each well. The use of a multi-channel pipet is recommended.
7. Swirl the plate rapidly to mix the contents and cover the wells with tape or Parafilm. Alternately, the plate may be incubated on a rotator for continuous mixing during incubation.
8. Incubate for 30 minutes.
9. After incubation, remove the covering and vigorously shake the contents of the wells into a sink. Flood the wells completely with cool running tap water, then shake to empty. Repeat this wash step four times for a total of five washes. Invert the plate on absorbent paper and tap out as much water as possible.
10. Add 100 µL of Substrate to each well.
11. Cover the wells and incubate for 10 minutes.
12. Add 100 µL of Stop Solution to each well in the same order of addition as the Substrate.
13. Measure the absorbance of each well at 450nm in a plate reader. It is recommended that the absorbance at 650nm be subtracted from the 450nm values.

Calculation

1. After you read all of the wells, average the OD of each set of calibrators, controls and samples, and calculate the %Bo as follows:
2. %B° = (average OD of calibrator, control or sample x 100) ÷ average OD of negative control.
3. 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.
4. Determine the Capsaicin concentration of each sample by finding its %Bo value and the corresponding concentration level on the graph.
5. 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.
6. Determine the capsaicin concentration of each sample using the standard curve. Multiply the number obtained from the standard curve (in ppm) by the extraction and dilution factors. Capsaicin (SU) = (conc. from curve)x (extraction factor)x (dilution factor)x (16 SU/ppm) Note: The 1:10 dilution in step 4 of the extraction procedure should not be factored into your calculations as the calibrators are also diluted 1:10 into PBS.

Typical Standard Curve

For a low heat sample determined to contain 0.028 ppm capsaicin using the standard curve.

[Capsaicin] = (0.028 ppm)(100)(10)(16 SU/ppm) = 448 SU

For a high heat sample determined to contain 0.1 ppm capsaicin using the standard curve.

[Capsaicin] = (0.1 ppm)(100)(1000)(16 SU/ppm) = 160,000 SU

Well Contents	OD	Average OD ± SD**	%RSD	%Bo	Capsaicin Conc. (ppb)
Negative Control	2.025 1.968	1.997 ± 0.04	2.02	100	N/A
1 ppb Calibrator	1.647 1.609	1.628 ± 0.022	1.65	81.5	N/A
10 ppb Calibrator	1.002 1.053	1.028 ± 0.036	3.51	51.5	N/A
100 ppb Calibrator	0.577 0.582	0.580 ± 0.004	0.61	29.0	N/A
Sample	0.836 0.844	0.840 ± 0.006	0.67	42.1	28.2

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

Precautions

1. Store all plate kit components at 4°C to 8°C (39°F to 46°F) when not in use.
2. Do not freeze plate kit components or expose them to temperatures greater than 37°C (99°F).
3. Allow all reagents and samples to reach ambient temperature before you begin the test.
4. Do not use plate kit components after the expiration date.
5. Do not mix reagents or test well strips from plate kits with different lot numbers.
6. Use approved methodologies to confirm any positive results.