

Intended Use

The Beacon Capsaicin HS Plate Kit is an immunoassay for the detection of Capsaicin in dehydrated peppers and oleoresin samples. This product is intended for research use only.

Principles

Calibrators and the Sample Extract(s) are pipetted into the test wells followed by Capsaicin HRP Enzyme Conjugate. 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 Unit Plate containing 12 test strips of 8 wells each that are vacuum sealed in an aluminized pouch with a desiccant.
- 1 X 2 mL Vial of Capsaicin Calibrator (0 ppb).
- 3 X 2 mL Vials of Capsaicin (Natural Mixture) Stocks (1, 10 and 100 ppb). Dilute prior to use.
- 1 X 12 mL Bottle of Capsaicin HRP Enzyme Conjugate.
- 1 X 25 mL Bottle of 10X Dilution Buffer. Dilute prior to use.
- 1 X 14 mL Bottle of Substrate.
- 1 X 14 mL Bottle of Stop Solution.

Reagents and Materials Required but Not Provided

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Positive displacement pipette(s) with disposable tips capable of dispensing the required volume(s).
- Multichannel pipette(s) (8 channels) with disposable tips capable of dispensing the required volume(s) (recommended if running more than two strips at once).
- Laboratory quality distilled or deionized water.
- Reagents and materials for sample preparation.
- Personal protective equipment.
- Paper towels or equivalent absorbent material.
- Wash bottle (optional).
- Timer.
- Microtiter plate or strip reader capable of reading at 450 nm.

Kit Handling Notes and Precautions

- Read the product brochure in its entirety prior to use.
- The kit, in its original packaging, can be used until the end of the month indicated on the box label.
- Do not use reagents after expiration date.
- Store all kit components at 2°C to 8°C (36°F to 46°F) when not in use.
- 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.
- Do not freeze kit components or expose them to temperatures greater than 37°C (99°F).
- Running Calibrators and Samples in duplicate will improve assay precision and accuracy.
- 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.
- If running more than two strips at once, the use of a multi-channel pipette is recommended when adding the Substrate and Stop Solution.
- All procedural steps should be completed without interruption. Ensure all reagents, materials and equipment are ready at the appropriate time.
- Each reagent is optimized for use in the Beacon Capsaicin HS Plate Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Capsaicin HS Plate Kits with different lot numbers.
- Do not reuse test wells.
- Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
- Damage to or obstruction of the optical surface may cause unsatisfactory results.

Performance Characteristics

The Beacon Capsaicin HS 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 ppb.

Compound	50% B ₀	% Cross-Reactivity
Capsaicin (Natural Mixture)*	12	100
Capsaicin (Pure)	11.5	104
Dihydrocapsaicin	12.25	98

*Contains approximately 65% Capsaicin and 35% Dihydrocapsaicin.

1X Dilution Buffer Preparation

1. Measure 225 mL of laboratory quality distilled or deionized water and transfer to a clean container with a tight-fitting lid.
2. Transfer the contents of the 10X Dilution Buffer bottle to the container.
3. Gently swirl to mix.

Sample Preparation

Dehydrated Peppers and Oleoresins:

1. Weigh 0.1 g of sample into a clean container with a tight fitting lid.
2. Measure 10 mL of 100% methanol and add to the container.
3. Tightly cap and incubate at room temperature using the appropriate protocol as follows:
Dehydrated Peppers: Incubate for 60 minutes. Vortex every 10-15 minutes.
Oleoresins: Incubate for 30 minutes. Vortex every 5-10 minutes.
4. Dilute the extract in 100% methanol using a positive displacement pipette and the appropriate protocol as follows:
Low Heat Variety Samples: Dilute 1:10
High Heat Variety Samples: Dilute 1:1000
5. Dilute the sample 1:10 in 1X Dilution Buffer for use in the assay.
Note: This dilution step should not be factored into sample concentration calculations since the calibrators are also diluted 1:10 in 1X Dilution Buffer.

Assay Procedure

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Prepare the calibrators by diluting the stocks 1:10 into 1X Dilution Buffer using a positive displacement pipette. Mix thoroughly.
Note: When stored properly at 2 – 8°C, the diluted calibrators can be used for the life of the kit. They do not need to be prepared fresh for each test.
3. Place the appropriate number of test wells into a holder. Be sure to re-seal unused test wells in the zip-lock bag with the desiccant to limit exposure to moisture.
4. Dispense **100 µL of Calibrators and Sample Extract(s)** into the appropriate well. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
5. Dispense **100 µL of Enzyme Conjugate** into each well.
6. Gently shake the wells for 30 seconds using a back-and-forth motion and incubate for **30 minutes** at room temperature.
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 µL 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 µL 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 (QC) Criteria

- 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) $< 20\%$.

Result Interpretation

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. Please contact Beacon for further details.

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

- Ensure QC criteria are met.
- 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.

Matrix	Heat Variety	Dilution Factor
Dehydrated Peppers and Oleoresins	Low	1,000
Dehydrated Peppers and Oleoresins	High	100,000

- In the event that the average absorbance of the sample is lower than the highest calibrator, further dilute the sample extract in 1X Dilution Buffer to fit into the standard curve and retest alongside the calibrators. Sample results must be multiplied by the total dilution factor used.
- To convert the Capsaicin concentration to Scoville Units (SU), multiply the Capsaicin concentration in ppm by 16 SU/ppm.

Technical Assistance

For questions regarding this kit or for additional information about Beacon products, contact us.

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Safety

Stop Solution is 1N hydrochloric acid. Handle with care. To receive complete safety information on this product, contact Beacon Analytical Systems, Inc., and request Safety Data Sheets.

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