CALCULATE RESULTS

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.

SAMPLE CALCULATIONS

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

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

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

Capsaicin] = (0.028 ppm)(100)(1000)(16 SU/ppm) = 44,800 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.

TECHNICAL ASSISTANCE

For questions regarding this kit or for additional information about Beacon products, call (207) 571-4302

SAFETY

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

General Limited Warranty

Beacon Analytical Systems, Inc. (“Beacon”) warrants the products manufactured by it against defects in materials and workmanship when used in accordance with the applicable instructions for a period not to extend beyond a product’s printed expiration date. BEACON MAKES NO OTHER WARRANTY, EXPRESSED OR IMPLIED. THERE IS NO WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The warranty provided herein and the data, specifications and descriptions of Beacon products appearing in published catalogues and product literature may not be altered except by express written agreement signed by an officer of Beacon. Representations, oral or written, which are inconsistent with this warranty or such publications are not authorized and, if given, should not be relied upon.

In the event of a breach of the foregoing warranty, Beacon’s sole obligation shall be to repair or replace, at its option, any product or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies Beacon promptly of any such defect. The exclusive remedy provided herein shall not be deemed to have failed of its essential purpose so long as Beacon is willing and able to repair or replace any nonconforming Beacon product or part. Beacon shall not be liable for consequential, incidental, special or any other indirect damages resulting from economic loss or property damage sustained by a customer from the use of its products. However, in some states the purchaser may have rights under state law in addition to those provided by this warranty.

BEACON ANALYTICAL SYSTEMS, INC.
82 Industrial Park Road
Saco, ME 04072
Tel. (207) 571-4302
Fax (207) 602-6502
www.beaconkits.com

Beacon Analytical Systems Inc.

Capsaicin HS Plate Kit

Cat.# 20-0027

Instructional Booklet

READ COMPLETELY BEFORE USE.

INTENDED USE

The Beacon Capsaicin Plate Kit is an immunological laboratory test for the quantitation of Capsaicin in Dehydrated Peppers and Oleoresins.

USE PRINCIPLES

The Beacon Capsaicin Plate 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

MATERIALS PROVIDED IN THE BEACON CAPSAICIN PLATE KIT

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

1 plate containing 12 strips of 8 wells coated with rabbit anti-Capsaicin 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

PERFORMANCE CHARACTERISTICS

The Beacon Capsaicin 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% B_o and the percent cross-reactivity (%CR) versus Capsaicin (natural). All concentrations are in parts per billion (ppb).

Compound	50% B_o	%CR
Capsaicin (natural mixture)*	12	100
Capsaicin (pure)	11.5	104
Dihydrocapsaicin	12.25	98

*Contains ~ 65 % capsaicin and 35 % dihydrocapsaicin

MATERIALS REQUIRED BUT NOT PROVIDED

- 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

PRECAUTIONS

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

EXTRACTION SOLUTION 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 5-10 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 30 mL vial of 10X PBS to 300mL 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.