

ChromaDex™

bioluminex

TECHNOLOGY
INVENTED
BY BAYER

www.bioluminex.cc

Bioluminex™ is a unique and patented bio-analytical tool that combines the simple analytical technique of thin-layer chromatography (TLC) with a biosensor detection method providing a unique and effective way of analyzing complex mixtures. Traditional bioassays have failed to adequately address problems associated with complex sample matrices and multiple analyte detection. The Bioluminex™ assay solves these issues by first separating complex mixtures into individual components and then simultaneously providing a chemical and biological profile of each constituent. Bioluminex™ offers all the advantages you have been searching for with related bioassays such as microtox.

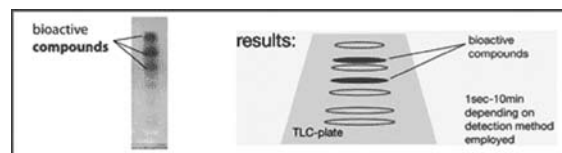
Simultaneously Analyze Complex Mixtures for Multiple Analytes

- Detects natural, biological, and chemical toxins.
- Detects chemical contaminants and pollutants.
- Identifies potential biological activity (i.e. anti-microbial screening).
- Supports raw material identity testing (chemical and biological profiling).
- Acquires characteristic chemical and biological profiles simultaneously.
- Parallel sample processing.
- Analyze up to 22 samples simultaneously.
- Detection can be qualitative and quantitative.
- Rapid, simple, and cost-effective kit format for up to 200 samples.



What is the Bioluminex™ method?

First, high performance thin-layer chromatography (HP-TLC) is used to separate multi-component mixtures into discrete substance zones. After compound separation the development solvent or mobile phase is evaporated and the HP-TLC plate is coated with a bioluminescent solution of *Vibrio fischeri* using a simple dipping procedure. Single toxic compounds are then selectively identified as dark zones (areas of inhibited bacteria) on a luminescent background (areas of viable bacteria). Results occur within seconds and last until the plate dries, approximately 30 minutes. Data can be documented using a cooled CCD camera with a dark box or x-ray film. Additionally data can be quantitated directly on the TLC plate using densitometry.



How does Bioluminex™ work?

The Bioluminex™ assay is an innovative application of two existing technologies. It couples the separation power of high performance, thin-layer chromatography (HP-TLC) with the biosensor properties of the bioluminescent marine organism *Vibrio fischeri*, a bacterium widely used for toxicity screening of water. TLC is an inexpensive and rapid technique that uses adsorption and capillary action to separate complex mixtures into discrete zones. This technique provides a characteristic distribution pattern of compounds that is dependent on sample composition. The TLC plate is then coated with the non-pathogenic bioluminescent bacteria, *Vibrio fischeri*, which emit light as a by-product of cellular respiration. As *Vibrio fischeri* cells reach a critical cellular density the bioluminescence reaction catalyst, luciferase is expressed. Luciferase, in the presence of oxygen, then catalyzes an oxidation reaction that releases excess energy in the form of light. This observed bioluminescence reflects the metabolic status of the cell and will decrease for cells exposed to toxic substances. Thus a reduction in light emission is a measure of toxicity that can be selectively viewed directly on the TLC plate.

- Complex mixtures are first separated into discrete zones by TLC.
- The TLC plate is then coated with the bioluminescent bacteria, *Vibrio fischeri*.
- Toxic compounds are easily identified as dark spots on a luminescent background.
- Degree of toxicity is proportional to luminescence inhibition.





Below is a list of a few relevant examples of both samples and analytes. This list is not comprehensive and is just included as guidance for the types of samples and analytes we have tested with Bioluminex™.

List of examples – for contamination and fingerprinting

- Mycotoxins (aflatoxin, ochratoxin, patulin)
- Polyaromatic Hydrocarbons (PAH) (Benzopyrene)
- Heavy Metals (Arsenic oxide, Mercury chloride)
- Pesticides (fungicides, herbicides, insecticides)
- Pharmaceuticals (Acetaminophen)
- Poisons (Strychnine, Monofluoroacetate)
- Marine Toxins (Domoic acid)
- Plant Toxins (Aristolochic acids, Ginkgolic acids)
- Phytochemicals (Capsaicin)
- Steroids (Andro)
- Chemicals
- Pollutants
- Plant Extracts



Application Notes:

Multiple Toxin Detection in Water

Assay: Bioluminex™

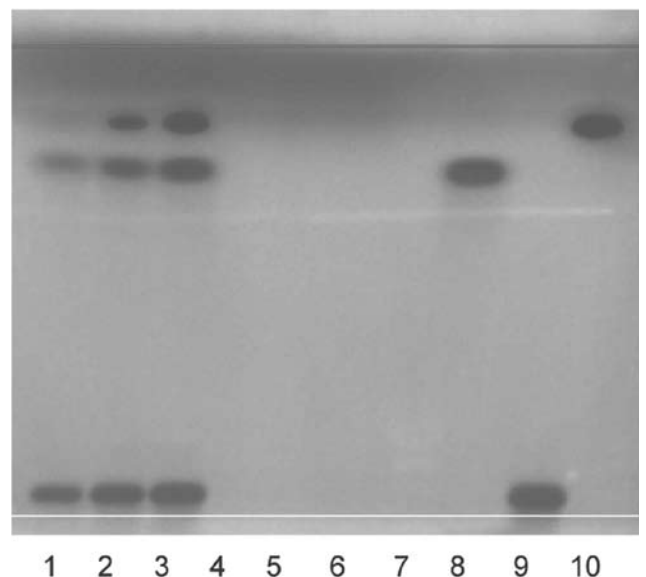
Samples: Tap water was spiked with arsenic oxide, metolachlor, and 4-androstene-3,17-dione and compared to unspiked tap water using the Bioluminex™ assay.

Mobile Phase: Toluene:ethyl acetate: formic acid: water (4:8:1.1:0.2)

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume	Analyte Amount (each)
1	Tap water-Toxins	8 µL	0.6 µg
2	Tap water-Toxins	14 µL	1.1 µg
3	Tap water-Toxins	20 µL	1.6 µg
4	Tap water	8 µL	N/A
5	Tap water	14 µL	N/A
6	Tap water	20 µL	N/A
7	Ascorbic Acid	2 µL	2 µg
8	Androstene	2 µL	2µg
9	Arsenic oxide	2 µL	2 µg
10	Metolachlor	2 µL	2 µg





Cedar leaf oil – Antimicrobial Activity

Assay: Bioluminex™

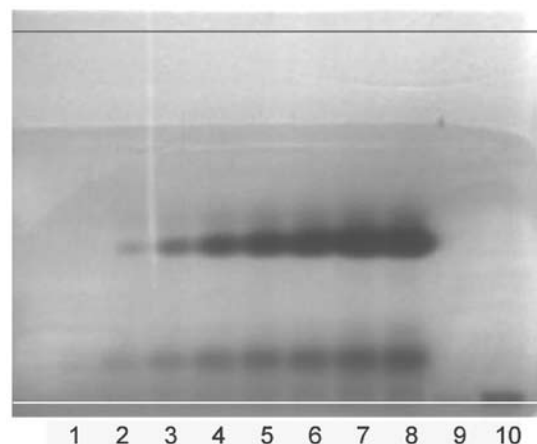
Samples: A diluted sample of cedar leaf oil was prepared via four serial dilutions (1:300 dilution) and assayed using the Bioluminex™ analysis.

Mobile Phase: Toluene:ethyl acetate (97:3)

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume
1	Ceaderleaf oil	0.2 µL
2	Ceaderleaf oil	0.5 µL
3	Ceaderleaf oil	1 µL
4	Ceaderleaf oil	2 µL
5	Ceaderleaf oil	3 µL
6	Ceaderleaf oil	4 µL
7	Ceaderleaf oil	6 µL
8	Ceaderleaf oil	8 µL
9	Negative control	8 µL, 8 µg
10	Positive control	8 µL, 4 µg



White zinfandel – Carbaryl

Assay: Bioluminex™

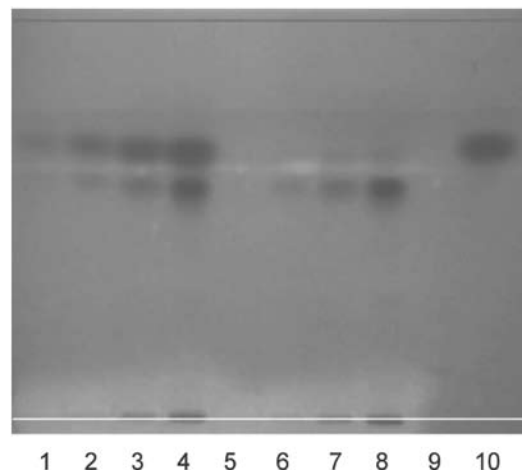
Samples: White zinfandel was spiked with carbaryl and compared to unspiked white zinfandel using Bioluminex™.

Mobile Phase: Toluene:ethyl acetate: formic acid: water (4:8:1.1:0.2)

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume	Analyte Amount
1	White zinfandel-carbaryl	2 µL	0.01 µg
2	White zinfandel-carbaryl	5 µL	0.05 µg
3	White zinfandel-carbaryl	10 µL	0.1 µg
4	White zinfandel-carbaryl	µL	0.2 µg
5	White zinfandel	µL	N/A
6	White zinfandel	µL	N/A
7	White zinfandel	µL	N/A
8	White zinfandel	µL	N/A
9	Ascorbic Acid	µL	4 µg
10	Carbaryl	µL	0.2 µg



Green tea – catechins

Assay: Bioluminex™

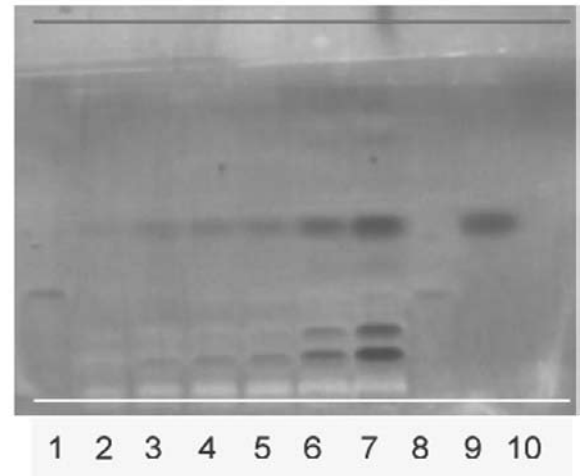
Samples: Bioluminex™ fingerprint of *Camellia sinensis*.

Mobile Phase: Chloroform:ethyl acetate:formic acid:methanol (5/4/2/2)

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume	Analyte Amount
1	(+)-catechin	2 µL	2.00 µg
2	Green Tea	1 µL	2.00 µg
3	Green Tea	2 µL	2.25 µg
4	Green Tea	3 µL	4.50 µg
5	Green Tea	2 µL	6.75 µg
6	Green Tea	5 µL	9.00 µg
7	Green Tea	10 µL	22.50 µg
8	(-)-epicatechin	2 µL	45.00 µg
9	Negative Control	8 µL	4.00 µg
10	Positive Control	4 µL	4.00 µg



Honey – Aflatoxin Detection

Assay: Bioluminex™

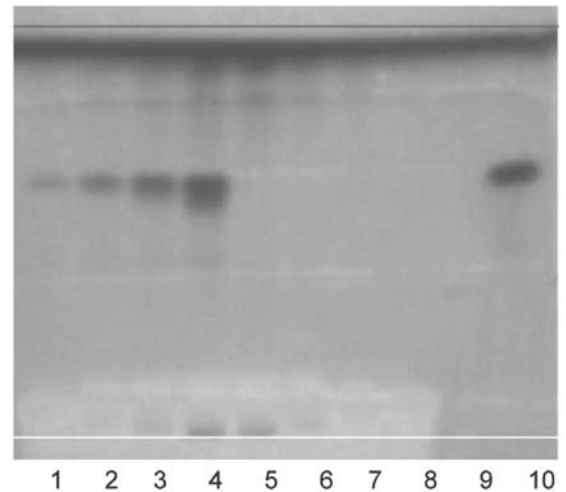
Samples: Honey was spiked with aflatoxin B1 and compared to unspiked honey using the Bioluminex™ assay.

Mobile Phase: Ethyl acetate: methanol: formic acid: water (50:2:5:3)

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume	Analyte Amount
1	Honey-Aflatoxin	2 µL	0.1 µg
2	Honey-Aflatoxin	5 µL	0.3 µg
3	Honey-Aflatoxin	10 µL	0.5 µg
4	Honey-Aflatoxin	20 µL	1.1 µg
5	Honey	2 µL	N/A
6	Honey	5 µL	N/A
7	Honey	10 µL	N/A
8	Honey	20 µL	N/A
9	Ascorbic Acid	4 µL	4 µg
10	Aflatoxin	2 µL	1 µg





Cayenne Pepper

Assay: Bioluminex™

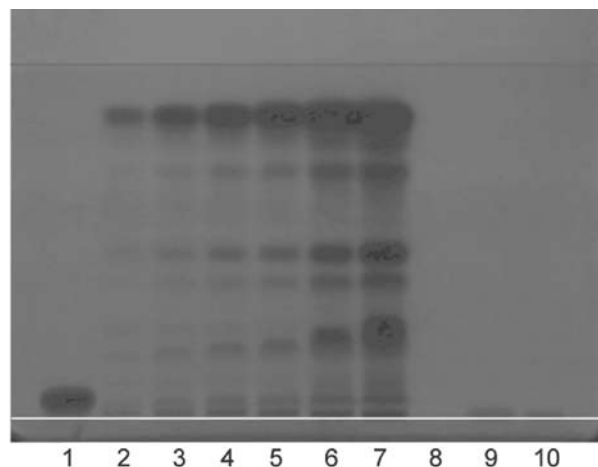
Samples: Bioluminex™ fingerprint analysis of cayenne pepper reference material

Mobile Phase: Isopropyl ether

Detection: FluorChem® 8900 Imaging System 2 minute exposure, 10 minute incubation.

Results:

Lane	Sample	Application Volume	Analyte Amount
1	Capsaicin	2 µL	2.00 µg
2	Cayenne Pepper Extraction 1:10 Dilution	1 µL	0.88 µg
3	Cayenne Pepper Extraction 1:10 Dilution	2 µL	1.75 µg
4	Cayenne Pepper Extraction 1:10 Dilution	3 µL	2.63 µg
5	Cayenne Pepper Extraction 1:5 Dilution	2 µL	3.50 µg
6	Cayenne Pepper Extraction 1:5 Dilution	5 µL	8.75 µg
7	Cayenne Pepper Extraction 1:5 Dilution	10 µL	17.50 µg
8	Empty	Empty	Empty
9	Caffeine	4 µL	4.00 µg
10	Ascorbic	2 µL	2.00 µg



Bioluminex Ordering Information

Bioluminex™ Kits:

Order #	Description	Unit
KIT-00002750-001	Bioluminex Standard Kit:	1 Kit
KIT-00002751-001	Bioluminex Hand Spot Kit	1 Kit
KIT-00002752-001	Bioluminex Standard Kit w/ X-ray Film	1 Kit
KIT-00002753-001	Bioluminex Hand Spot Kit w/ X-ray Film	1 Kit
KIT-00002754-001	Bioluminex Standard Kit without TLC Plates	1 Kit

Individual Bioluminex™ Kit Items:

Order #	Description	Unit
ASB-00002700-001	Bioluminex Bacteria A	1 Vial
ASB-00002710-040	Bioluminex Media A	40g
ASB-00002711-005	Bioluminex Media B	5.5g
ASB-00002712-085	Bioluminex Buffer A	85g
ASB-00002725-020	Bioluminex HPTLC Plates 10x10, Si60F254	20 plates
ASB-00002726-020	Bioluminex HPTLC Plates 10x10, Si60F254 w/ Preconc zone	20 plates
ASB-00002735-025	Bioluminex Positive Control Standard	25mg
ASB-00002736-025	Bioluminex Negative Control Standard	25mg
ASB-00002727-001	Drummond Micropipette Spotting Kit 100 caps	1 Box
ASB-00002728-025	Bioluminex X-ray Film 5"x7"	25 sheets



Bioluminex™ Kit Contents:

Order #	Kit
KIT-00002750-001	Bioluminex Standard Kit Contains: <ol style="list-style-type: none"> 10 x Bioluminex Bacteria A Vials 2 x Bioluminex Media A 40g 2 x Bioluminex Media B 5.5g 2 x Bioluminex Buffer A 85g 1 x Bioluminex HPTLC Plates 10x10, Si60F254 1 box of 20 plates 1 x Bioluminex Positive Control Standard 25mg 1 x Bioluminex Negative Control Standard 25mg
KIT-00002751-001	Bioluminex Hand Spot Kit Contains: <ol style="list-style-type: none"> 10 x Bioluminex Bacteria A Vials 2 x Bioluminex Media A 40g 2 x Bioluminex Media B 5.5g 2 x Bioluminex Buffer A 85g 1 x Bioluminex HPTLC Plates 10x10, Si60F254 Preconc Zone 1 box of 20 plates 1 x Bioluminex Positive Control Standard 25mg 1 x Bioluminex Negative Control Standard 25mg 2 x Drummond Micropipette Spotting Kit 100 caps
KIT-00002752-001	Bioluminex Standard Kit w/ X-ray Film Contains: <ol style="list-style-type: none"> 10 x Bioluminex Bacteria A Vials 2 x Bioluminex Media A 40g 2 x Bioluminex Media B 5.5g 2 x Bioluminex Buffer A 85g 1 x Bioluminex HPTLC Plates 10x10, Si60F254 1 box of 20 plates 1 x Bioluminex Positive Control Standard 25mg 1 x Bioluminex Negative Control Standard 25mg 1 x Bioluminex X-ray Film 5"x7" 1 box of 25 sheets
KIT-00002753-001	Bioluminex Hand Spot Kit with X-ray Film Contains: <ol style="list-style-type: none"> 10 x Bioluminex Bacteria A Vials 2 x Bioluminex Media A 40g 2 x Bioluminex Media B 5.5g 2 x Bioluminex Buffer A 85g 1 x Bioluminex HPTLC Plates 10x10, Si60F254 Preconc Zone 1 box of 20 plates 1 x Bioluminex Positive Control Standard 25mg 1 x Bioluminex Negative Control Standard 25mg 1 x Bioluminex X-ray Film 5"x7" 1 box of 25 sheets 2 x Drummond Micropipette Spotting Kit 100 caps
KIT-00002754-001	Bioluminex Standard Kit Without TLC Plates Contains: <ol style="list-style-type: none"> 10 x Bioluminex Bacteria A Vials 2 x Bioluminex Media A 40g 2 x Bioluminex Media B 5.5g 2 x Bioluminex Buffer A 85g 1 x Bioluminex Positive Control Standard 25mg 1 x Bioluminex Negative Control Standard 25mg

