**Result Interpretation**

Semi-Quantitative Interpretation: Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrators:

- Samples with a lower absorbance (less color) than a calibrator have a concentration of Spinosyn greater than the concentration of the calibrator.
- Samples with a higher absorbance (more color) than a calibrator have a concentration less than the concentration of the calibrator.

Quantitative 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.

- The concentration of Spinosyn 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.
- Samples with absorbances lower than the highest calibrator contain a concentration of Spinosyn too high for quantification. Further dilute the sample extract in 1X surfactant solution to fit into the standard curve and retest along with the calibrators. Results must then be multiplied by the dilution factor used.
- Samples with Spinosyn absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.05 ppb or > 0.5 ppb, respectively.

**Technical Assistance**

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

Beacon Analytical Systems, Inc.
82 Industrial Park Rd. Saco, ME 04046
Tel. 207-571-4302
info@beaconkits.com | www.beaconkits.com

**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.

**General Limited Warranty**





**Intended Use**

The Beacon Spinosyn 100 Tube Kit is an immunoassay for the detection of Spinosyn in water samples. This product is intended for research use only.

**Principles**

Calibrators and the Sample Extract(s) are pipetted into the test tubes followed by a soluble polyclonal Spinosyn Antibody solution. After an incubation, Spinosyn HRP Enzyme Conjugate is added to each tube. During an incubation, Spinosyn and Spinosyn HRP Enzyme Conjugate compete for binding to the soluble Spinosyn Antibody which is in turn immobilized on the test tubes. Following this incubation, the tubes are washed to remove any non-specific binding. After washing, a colorless substrate is added to the tubes 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 tube is measured. The color of the unknown sample is compared to the color of the calibrators and the Spinosyn concentration of the sample is derived.

**Reagents and Materials Provided**

5 Bags each containing 20 test tubes that are vacuum sealed in an aluminized pouch with a desiccant.
5 Vials of Spinosyn Calibrators (0, 0.05, 0.125, 0.25, and 0.5 ppb).
1 Bottle of Spinosyn HRP Enzyme Conjugate.
1 Bottle of Spinosyn Antibody.
1 Bottle of Substrate.
1 Bottle of Stop Solution.

**Reagents and Materials Required but Not Provided**

- Pipette(s) with disposable tips capable of dispensing the required volume(s).
- Repeater pipette(s) with disposable tips capable of dispensing the required volume(s) (recommended if running more than five tubes 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).
- Permanent Marker.
- Tube rack.
- Timer.
- Photometer capable of reading absorbance at 450 nm in 12 mm x 75 mm tubes.

**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 4°C to 8°C (39°F to 46°F) when not in use.
- Reagents should be brought to room temperature, 20°C to 28°C (62°C 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 five tubes at once, the use of a repeater pipette is recommended when adding the Antibody, 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 Spinosyn 100 Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other Beacon Spinosyn 100 Tube Kits with different lot numbers.
- 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.

**Specificity**

| | Compound | | % Cross-Reactivity |
|---|---|---|---|
| TSN-102499 | Spinosyn A | XDE-105 | 95% |
| TSN-101600 | Spinosyn D | DE-105 | 111% |
| TSN-101123 | DE-105 Factor L | N/A | 75% |
| TSN-104472 | XDE-175 | XR-175 | 100% |
| TSN-105089 | XDE-175 | X574175 | 87% |
| TSN-104480 | Factor L | X-513999 | 125% |
| TSN-105125 | N-formyl-XDE-175-L | X11365311 | |
| TSN-105115 | N-formyl-XDE-175-J | X11360594 | |
| TSN-105268 | 2'-demethyl XDE-175-L | X11396623 | 96% |
| TSN-105269 | 2'-demethyl XDE-175-J | X11396622 | 149% |
| TSN-105124 | N-demethyl XDE-175-L | X11318312 | 0.70% |
| TSN-105114 | N-demethyl XDE-175-J | X573006 | 3% |
| TSN-100724 | N-demethyl Spinosyn D | N/A | 0.75% |
| TSN-104739 | 5,6-dihydro-Spinosyn Factor J | X516842 | 92% |
| TSN-102302 | DE-105 Factor B | N/A | 1.40% |

**Precision**

| Calibrator Concentration (ppb): | 0.3 | 0.1 |
|---|---|---|
| Replicates: | 3 | 3 |
| Days: | 2 | 2 |
| N: | 9 | 9 |
| Mean (ppb): | 0.310 | 0.104 |
| % CV (within the assay): | 2.31 | 2.83 |
| % CV (between the assays): | 3.95 | 0.25 |
| Max % from Theoretical: | 11.2 | 6.98 |

**Sensitivity**

The Beacon Spinosyn 100 Tube assay has a calculated Least Detectable Dose (LDD) of 0.014 ng/mL for XDE-175 which is the compound used in the calibrators.

**Sample Preparation**

Water: (Dilution Factor: 1.01)

1. Collect the sample in a clean glass or plastic container with a tight-fitting lid.
2. Immediately add 1 mL of 100X surfactant solution per 100 mL of sample. This will reduce the absorption of Spinosyn to the glass or plastic surface of the collection container.
3. Gently swirl to mix and use in the assay.

**Assay Procedure**

1. Allow kit components and the sample extract(s) to reach room temperature prior to running the test.
2. Place the appropriate number of test tubes into a tube rack. Label the tubes one inch from the top with the calibrator concentration or sample identification. Be sure to re-seal unused tubes in the zip-lock bag with the desiccant to limit exposure to moisture.
3. Dispense **500 µL of Calibrators and Sample Extract(s)** into the appropriate tube. Be sure to use a clean pipette tip for each solution to avoid cross contamination.
4. Dispense **250 µL of Antibody** into each tube.
5. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **15 minutes** at room temperature.
6. Dispense **250 µL of Enzyme Conjugate** into each tube.
7. Gently shake the tubes for 30 seconds using a back-and-forth motion and incubate for **15 minutes** at room temperature.
8. Decant the contents of the tubes into an appropriate waste container. Fill the tubes to overflowing with laboratory quality distilled or deionized water and then decant. Repeat this wash step three times for a total of four washes. Following the last wash, tap the inverted tubes onto absorbent paper to remove excess wash solution.
9. Dispense **500 µL of Substrate** into each tube.
10. Incubate for **15 minutes** at room temperature.
11. Dispense **500 µL of Stop Solution** into each tube in the same order of addition as the Substrate.
12. Gently shake the tubes for 30 seconds using a back-and-forth motion.
13. Carefully wipe the optical surface with a soft, lint-free wipe. Measure and record the absorbance (Optical Density; OD) of each tube at 450 nm using a tube reader within 10 minutes of stopping the assay. Be sure to blank the reader with laboratory quality distilled or deionized water prior to measuring.