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ABRAXIS® Spinosyn ELISA Tube

Product No. 50020B

Intended Use

The ABRAXIS® Spinosyn ELISA Tube Kit is a competitive ELISA for the quantitative analysis of Spinosyn in water samples.

Assay Principles

The ABRAXIS® Spinosyn ELISA Tube Kit is a competitive enzyme-labeled immunoassay. The sample is mixed with specific Spinosyn antibodies in a test tube that has been previously coated with a second antibody (GAR). After the initial 15 minute incubation of Antibody with sample, enzyme-labeled Spinosyn is added to the tube. The toxin from the extract and the enzyme-labeled toxin compete for a limited number of antibody binding sites. Following this second 15 minute incubation, the contents of the tubes are removed and the tubes are washed to remove any unbound toxin or enzyme-labeled toxin. A clear substrate is then added to the tubes and any bound enzyme-toxin conjugate causes the conversion to a blue color. Following a 15 minute incubation, the reaction is stopped and amount of color in each tube is read. The color of unknown samples is compared to the color of the calibrators and the Spinosyn concentration of the samples is derived.

Procedural Notes and Precautions

1. Each reagent is optimized for use in the ABRAXIS® Spinosyn ELISA Tube Kit. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other kits with different lot numbers.
2. Dilution or adulteration of reagents or samples not called for in the procedure may result in inaccurate results.
3. Do not use reagents after expiration date.
4. Reagents should be brought to room temperature, 20 – 28ºC (62 – 82ºF) prior to use. Avoid prolonged (> 24 hours) storage at room temperature.
5. Spinosyn is a toxic substance. Dispose of all liquids in an appropriate manner.
6. The Stop Solution is 1N hydrochloric acid. Avoid contact with skin and mucous membranes. Immediately clean up any spills and wash area with copious amounts of water. If contact should occur, immediately flush with copious amounts of water.

Working Instructions

Materials Provided

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. 40 anti-Rabbit IgG (capture antibody) coated test tubes
2. (5) Spinosyn calibrators (0, 0.05, 0.125, 0.25 and 0.5 ppb), 4 mL
3. Spinosyn-HRP Enzyme Conjugate, 12 mL
4. Anti-Spinosyn antibodies, 12 mL
5. Substrate, 24 mL
6. Stop Solution, 24 mL (Caution! 1N HCl. Handle with care.)
7. 100X surfactant solution (sample diluent), 10 mL

Material Required (not provided)

1. Laboratory quality distilled or deionized water.
2. Pipet with disposable tips capable of dispensing 250 and 500 µL.
3. Paper towels or equivalent absorbent material.
4. Photometer capable of reading 12mm tubes at 450nm.
5. Timer
6. Balance

Sample Preparation

1. Spinosyn adsorbs to glass and plastic surfaces. Immediately upon collection of sample, add 1mL 100X surfactant solution per 100 mL sample.
2. Samples containing >0.5 ppb Spinosyn will need to be diluted to obtain correct results. Dilution is recommended using the 1X surfactant solution.

Assay Procedure

1. Allow reagents and sample extracts to reach room temperature prior to running the test.
2. Place the appropriate number of capture antibody-coated tubes into the tube holder. Be sure to re-seal unused tubes in the zip-lock pouch with desiccant.
3. **Add 500 µL of each Calibrator and Sample Extract** into the appropriate tubes. Use a clean pipet tip for each.
4. **Add 250 µL of Antibody solution** into each tube.
5. **Vortex for 3-5 seconds**.
6. **Incubate 15 minutes at room temperature**.
7. **Add 250 µL of Enzyme Conjugate** into each tube.
8. **Vortex for 3-5 seconds**.
9. **Incubate the tubes for 15 minutes at room temperature**.
10. Dump the contents of the tubes into an appropriate waste container. **Fill the tubes to overflowing with laboratory grade water and dump wash. Repeat 4X** for a total of five washes.
11. Following the last wash tap the inverted tubes onto absorbent paper to remove the last of the wash.
12. **Add 500 µL of Substrate** into each tube.
13. **Vortex for 3-5 seconds**.
14. **Incubate the tubes for 15 minutes at room temperature**.
15. **Add 500 µL of Stop Solution** into each test tube.
16. Read and record the absorbance of the tubes at 450nm.

Results

1. Semi-quantitative results can be derived by simple comparison of the sample absorbances to the absorbance of the calibrator tubes: Samples containing less color than a calibrator tube have a concentration of Spinosyn greater than the concentration of the calibrator. Samples containing more color than a calibrator tube have a concentration less than the concentration of the calibrator.
2. Quantitative interpretation requires graphing the absorbances of the calibrators (X axis) versus the log of the calibrator concentration (Y axis) on semi-log graph paper. A straight line is drawn through the calibrator points and the sample absorbances are located on the line. The corresponding point on the Y-axis is the concentration of the sample. Samples with absorbances greater than the lowest calibrator or less than the highest calibrator must be reported as < 0.05 ppb or >0. 5 ppb, respectively.

Alternatively, Gold Standard Diagnostics can supply a spreadsheet template, which can be used for data reduction. Please contact Gold Standard Diagnostics for further details

Performance Data

Specificity

Compound	IC 85%		IC 50%		IC 20%	
	ppb	%CR	ppb	%CR	ppb	%CR
Spinosyn J	0.021	100.0	0.075	100.0	0.297	100.0
Spinosyn A	0.031	67.7	0.091	82.1	0.310	96.0
Spinosyn D	0.023	90.0	0.088	84.5	0.386	77.0
Spinosyn L	0.026	81.8	0.086	86.5	0.354	84.1
XDE-175-J	0.023	92.6	0.075	100.0	0.263	113.1
XDE-175-L	0.026	81.8	0.092	81.2	0.367	80.9
2'-demethyl XDE-175-L	0.026	80.8	0.089	83.6	0.352	84.5
2'-demethyl XDE-175-J	0.020	106.8	0.073	101.8	0.277	107.2
5,6-dihydro-Spinosyn J	0.025	85.1	0.075	99.6	0.258	115.2

Sensitivity

The spinosyn tube assay has a calculated Least Detectable Dose (LDD) of 0.014 ng/mL for XDE-175, which is the compound used for the standards.

Reproducibility

The standard curve R^2 must be ≥ 0.98. The Standard 0 absorbance value should be between 0.8 - 3.000

Precision

	0.3ppb	0.1ppb
Replicates	3	3
Days	2	2
n	9	9
Mean (ppb)	0.310	0.104
% CV (intra-assay)	2.31	2.83
% CV (inter-assay)	3.95	0.25
Max % from theoretical	11.2	6.98