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Technical Data Sheet

For research use only

Not intended or approved for diagnostic or therapeutic use.

Product Name: Sphingosine 1 Phosphate Assay Kit

Product Number: K-1900

Kit Components:

- Coated Microtiter plate (Nunc, Maxisorp sealed in plastic bag)
- Block Solution
- Microtiter plate seal (2)
- PBS Tablets
- Sphingosine 1 Phosphate (S1P) Standard
- Anti-S1P Antibody
- Anti-S1P Diluent
- Delipidized Human Sera
- Mixing plate (Costar not sealed in plastic bag)
- Streptavidin HRP
- TMB Substrate
- 1N Sulfuric Acid

Each kit provides sufficient reagents for 96 assays (including standards).

Researcher must provide:

Absorbance microtiter plate reader capable of reading at 450 nm.
Pipettes (20 μ L, 200 μ L, and 1,000 μ L)

Storage:

Upon receipt, the kit should be stored at -20°C protected from light. The TMB substrate, K-TMB1, needs to be removed from the kit and stored at 4°C . Under proper storage conditions, the kit components should remain stable for at least 6 months from date of receipt. Allow the reagents to warm to room temperature before opening vials.

Health Hazard Data:

The toxicity effects of delipidized human sera has not been determined. The kit contains human serum (in the form of delipidized human sera) and should be handled as potentially bio-hazardous materials. The serum was derived from blood donors tested individually and shown by FDA approved methods to be negative for antibodies to Human Immunodeficiency Virus 1/2 (HIV) and Hepatitis C Virus (HCV), non-reactive to Human Immunodeficiency Virus 1 (HIV1), Hepatitis B surface antigen (HBsAg) and Sexually Transmitted Diseases (RPR). Since no test method can offer complete assurance that HIV, Hepatitis B and C Virus or other infectious agents are absent, these products should be handled following all universal precautions.

Background:

Sphingosine 1 Phosphate (S1P) is a key component of the sphingolipid signaling cascade. S1P initiates a proliferative, pro-angiogenic, and anti-apoptotic sequence of events contributing to cancer progression. Recent research has suggested that S1P is a potent tumorigenic growth factor that is likely released from tumor cells and that S1P may be a novel biomarker for early stage cancer detection. Sphingosine kinase has also been

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TDS K-1900 Rev: 1 (7/06/07)

shown to be up-regulated in a variety of cancer types (S1P is produced via the activity of sphingosine kinase phosphorylating sphingosine). The Echelon S1P Assay is a sensitive and robust method for the quantification of S1P in a 96-well plate format.

Assay Procedure:

1. Remove coated microtiter plate (Nunc, Maxisorp) from plastic bag.
2. Block each well of microtiter plate by adding 150 μL of block solution per well and incubate at room temperature for 1 hour (place microtiter plate seal on plate).
3. Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.
4. Prepare standard dilutions of S1P Standard as follows (use standard or 1.5 mL polypropylene tubes):

μM S1P [final]	μL 100 μM stock or previous dilution	μL diluent (Delipidized Human Sera)
2 μM	12 μL (100 μM S1P Stock Soln.)	588 μL
1 μM	300 μL (2 μM Solution)	300 μL
0.5 μM	300 μL (1 μM Solution)	300 μL
0.25 μM	300 μL (0.5 μM Solution)	300 μL
0.13 μM	300 μL (0.25 μM Solution)	300 μL
0.06 μM	300 μL (0.13 μM Solution)	300 μL
0 μM	-	300 μL

5. Dilute anti-S1P antibody in anti-S1P diluent by adding 4 mL anti-S1P diluent to the bottle containing the anti-S1P antibody and mix well.
6. Dilute samples 1:10 in delipidized human sera (30 μL sample : 270 μL delipidized human sera). Combine with diluted anti-S1P antibody and mix in the mixing plate (Costar) before being transferred to the coated, blocked, and washed Microtiter plate (Nunc, Maxisorp). If replicates other than duplicate are preferred add the diluted antibody to the diluted sample 1:4.
 - a. Add 63 μL of diluted antibody to each well of the mixing plate (see plate layout below).
 - b. Add 188 μL of standard or diluted sample to each well of the mixing plate (see plate layout below).

Mixing Plate Layout (Costar)

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 μM	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
B	1 μM	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
C	0.5 μM	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
D	0.25 μM	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
E	0.13 μM	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
F	0.06 μM	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
G	0 μM	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-
H	-Blank-	Sample 8	Sample 16	Sample 24	Sample 32	Sample 40	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-	-Empty-

7. Place mixing plate on plate shaker for 30 seconds; or carefully tap the plate to mix.
8. Add 100 μL of mixture from the mixing plate to each well according to plate layout of the coated, blocked, and washed microtiter plate (Nunc, Maxisorp).

Suggested microtiter plate layout (Nunc, Maxisorp)

Samples could be run in single, duplicate, or triplicate as desired. -Duplicate shown-

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 μM	2 μM	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
B	1 μM	1 μM	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
C	0.5 μM	0.5 μM	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
D	0.25 μM	0.25 μM	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36

E	0.13 μ M	0.13 μ M	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
F	0.06 μ M	0.06 μ M	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38
G	0 μ M	0 μ M	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31	Sample 39	Sample 39
H	-Blank-	-Blank-	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32	Sample 40	Sample 40

- Incubate plate at 27 °C for one hour.
- Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.
- Dilute streptavidin HRP in block solution by adding 12.5 mL block solution to the streptavidin-HRP bottle (streptavidin HRP bottle contains 2.75 μ L streptavidin-HRP at 1 mg/mL) and mix well.
- Add 100 μ L of diluted streptavidin HRP to each well of the coated microtiter plate (Nunc, Maxisorp) and incubate at 27 °C for one hour. (place fresh microtiter plate seal on plate)
- Wash plate with PBS four times (4X) and on the final wash incubate the plate for 2 minutes before removing the wash solution. Ensure all wash buffer is removed before progressing to the next step.
- Add 100 μ L TMB substrate to each well of the microtiter plate (Nunc Maxisorp) and incubate for 4 minutes.
- Add 50 μ L H₂SO₄ to stop reaction.
- Read plate at 450 nm.

Spingosine 1 Phosphate

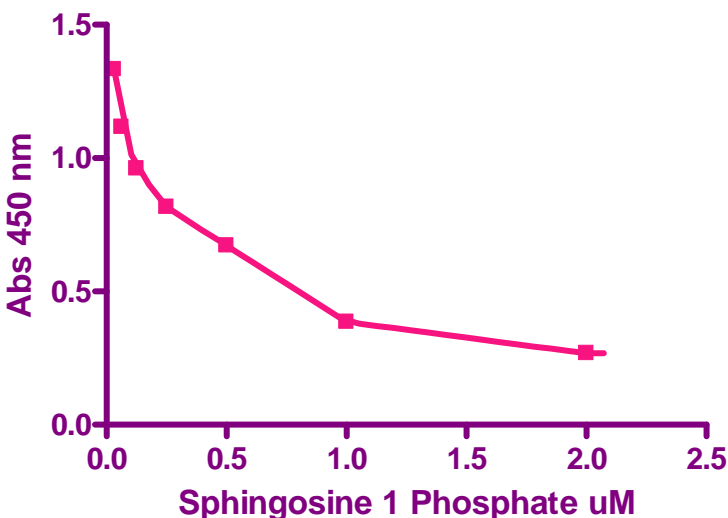


Figure. S1P competitive ELISA standard curve was graphed using point to point analysis. As little as 0.06 μ M can be detected in 100 μ L of sample.

Assay Notes:

- Ensure all samples are free from debris before adding to the plate.
- Use care when washing microtiter plate. Inconsistent microtiter plate washing will result in higher assay variation.
- All samples and standards must be diluted in delipidized human sera before use in the assay.
- Be cautious of edge effects. Wells at the edge of the microtiter plate may exhibit increased variation from wells on the interior of the plate. If concerned about edge effect or observe increased variation in the plate exterior wells do not use wells on the exterior of the microtiter plate.
- Diluted antibody and streptavidin HRP are stable at 4 °C for 3 days and at -20 °C for 3 weeks.
- Ensure bottom of plate is clean before reading absorbance (wipe off smudges and debris).
- Use caution when using delipidized human sera. Treat delipidized human sera as a potential biohazard and dispose of accordingly.