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Protocol for Sphingosine 1-Phosphate Lyase Activity Detection

This protocol uses Echelon's Sphingosine 1-Phosphate (Catalog No. S-2000) as a substrate and Sphingosine 1-Phosphate ELISA Kit (Catalog No. K-1900) for detection. We recommend using this protocol as a guideline. Optimize the protocol to fit your needs.

Materials:

- Sphingosine 1-Phosphate Lyase samples to be tested
- Sphingosine 1-Phosphate ELISA Kit (Catalog No. K-1900)
- Sphingosine 1-Phosphate, S1P (Catalog No. S-2000)
- 0.5 M Potassium Phosphate Buffer pH 7.4
- 5 mM Pyridoxal 5' Phosphate
- 0.5 M NaF
- 1% Triton X-100
- 1 M Dithiothreitol (DTT)
- Methanol
- Nanopure water

Protocol:

1. Aliquot 200 nmol S1P (Catalog No. S-2000) into 4 mL or larger glass vial according to the technical data sheet using methanol. SpeedVac to dry. Ensure S1P is completely dried without any methanol residue. Store aliquot at -20 °C until use.
2. Prepare 4 mL of S1P Substrate Mixture at 1.5X by adding the following reagents sequentially into the glass vial containing the 200 nmol S1P prepared in step 1. Vortex to mix. Keep the S1P Substrate Mixture at 37 °C until use.
   a. 500 µL of 1% Triton X-100. **Immediately vortex to mix before adding the next reagent.** Dried S1P should be completely dissolved after vortexing.
   b. 1000 µL of 0.5 M potassium phosphate buffer, pH 7.4
   c. 50 µL of 100 mM EDTA, pH 8.0
   d. 250 µL of 0.5 M NaF
   e. 5 µL of 1 M DTT (discard after use)
   f. 250 µL of 5 mM pyridoxal 5' phosphate (yellow in color)
   g. 1945 µL of Nano pure water
   - **S1P Substrate Mixture (1.5X)**: 50 µM S1P in 0.125 M Potassium Phosphate Buffer, pH 7.4 containing 0.125% Triton X-100, 1.25 mM EDTA, 31.25 mM NaF, 1.25 mM DTT, 0.3125 mM pyridoxal 5' phosphate
3. If needed, dilute the S1P lyase samples to be tested with the buffer of your choice, e.g. the same buffer used in S1P lyase sample preparation. Add 40 µL of samples into microcentrifuge tubes. Keep on ice until use. For negative control, add 40 µL of the buffer of your choice.
4. Bath sonicate the S1P Substrate Mixture prepared in step 2 on ice for 1 minute.
5. Start the S1P lyase reaction by adding 160 µL of the sonicated S1P Substrate Mixture (prepared in step 4) into each microcentrifuge tube containing 40 µL of S1P lyase sample (step 3).
   - **S1P Lyase Reaction (1X)**: S1P Lyase incubated with 40 µM S1P in 0.1 M Potassium Phosphate Buffer, pH 7.4 containing 0.1% Triton X-100, 1 mM EDTA, 25 mM NaF, 1 mM DTT, 0.25 mM pyridoxal 5' phosphate
6. Pulse vortex each reaction for 1 second to mix. Incubate reactions at 37 °C for desire time with gentle shaking. Stop reactions by freezing the reactions at -80 °C.
   - For kinetic measurements, take out 10 µL of reaction at each desired time point and freeze reaction at -80 °C. Continue incubate the remaining reactions at 37 °C with shaking until all time points are collected. 200 µL is enough for 8 time points. If needed, adjust the reaction volume. This can be done in the Yellow U-bottom Mixing Plate provided in the S1P ELISA (cat. No. K-1900).
7. Bring the S1P ELISA (Catalog No. K-1900) reagents to room temperature before use. Keep the Streptavidin HRP (Catalog No. K-SEC3h) and Anti-S1P Antibody (Catalog No. K-1901) on ice.
8. Thaw the collected S1P Lyase reaction samples. Immediately after thawing, dilute 10 µL of each reaction sample with 190 µL Delipidized Human Serum (Catalog No. K-1904). Keep on ice.
9. Then, follow the S1P ELISA (Catalog No. K-1900) technical data sheet to complete the ELISA.

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10. Interpolate samples against the S1P Standard curve.
11. S1P lyase activity can be calculated as follows:
    a. Amount of S1P depleted by S1P Lyase (ρmol): (ρmol of S1P Detected from Negative Control – ρmol of S1P Detected from Reaction Sample) x 20 (Sample Dilution Factor)
    b. SPL Lyase Activity (ρmol/µg/min): Amount of S1P depleted by S1P Lyase (ρmol) ÷ Amount of S1P Lyase in Reaction (µg) ÷ Reaction Incubation Time (min)

References

1. Weiler, S.; Braendlin, N.; Beerli, C.; Bergsdorf, C.; Schubart, A.; Srinivas, H.; Oberhauser, B.; Billich, A., Orally active 7-substituted (4-benzylphthalazin-1-yl)-2-methylpiperazin-1-yl]nicotinonitriles as active-site inhibitors of sphingosine 1-phosphate lyase for the treatment of multiple sclerosis. J Med Chem 2014, 57 (12), 5074-84.
2. Bedia, C.; Camacho, L.; Casas, J.; Abad, J. L.; Delgado, A.; Van Veldhoven, P. P.; Fabrias, G., Synthesis of a fluorogenic analogue of sphingosine-1-phosphate and its use to determine sphingosine-1-phosphate lyase activity. Chembiochem 2009, 10 (5), 820-2.

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