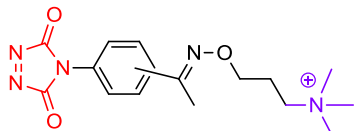




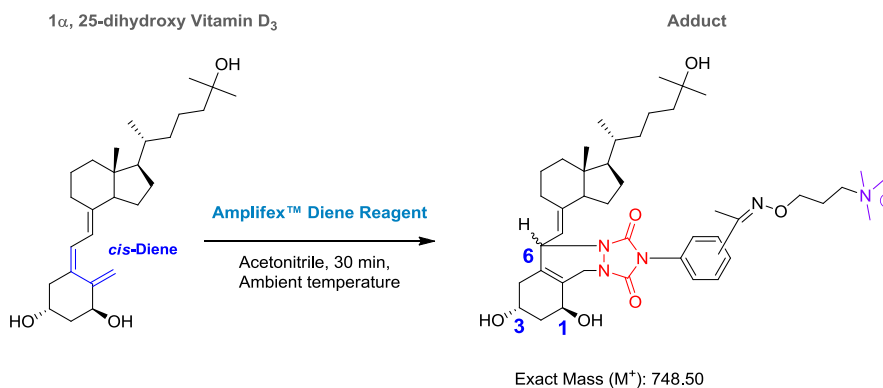
Amplifex™ Diene Reagent Kit

Chemistry

Amplifex™ Diene Reagent



Amplifex™ Diene Reagent reacts **specifically** with *cis*-Diene within 30 minute at ambient temperature to form an adduct. The reagent design enables very low limit of detection and quantitation for molecules that are difficult to detect by MS.



The kit includes the following components

1. Amplifex™ Diene Reagent (1 Tube containing 1.5-2.0 mg)
2. Amplifex™ Diene Diluent (1 Tube containing 3 mL)



Warning! CHEMICAL HAZARD. Before handling any chemicals read the safety data sheet (SDS). Always follow the safety precautions (wearing appropriate protective eyewear, clothing, gloves, etc.) presented in each SDS. SDSs may be found at www.sciex.com.

Labeling of Standard Analyte and LC/MS optimization

Prior to method optimization please ensure that the instrument tuning and calibration is valid.

For method optimization, a concentrated solution of the desired analyte is needed. The following describes the derivatization procedure:



1. Transfer approximately 3 μg of the analyte from a concentrated solution in solvent into a polypropylene microcentrifuge tube (e.g. for a 2 mg/mL solution transfer 1.5 μL).
2. Dry the solvent using vacuum or nitrogen solvent evaporation system.
3. Add 50 μL of Amplifex™ Diene Reagent solution (see below for preparation procedure).
4. Vortex mix and allow to derivatize for 30 min. at ambient temperature
5. Add 2950 μL of diluent (50% MeOH/50% H₂O (v/v), not provided in the kit) to make a 1 $\mu\text{g}/\text{mL}$ solution.
6. Dilute the derivatized analyte 5 fold to a final concentration of 0.2 $\mu\text{g}/\text{mL}$ with 50% MeOH/ 50% H₂O (v/v). The remaining of the 1 $\mu\text{g}/\text{mL}$ stock solution can be stored at -20°C.
7. Infuse the 0.2 $\mu\text{g}/\text{mL}$ solution directly into the ESI source at 10 $\mu\text{L}/\text{min}$ flow rate, using a syringe pump.

For best results "T" infuse the sample while the desired mobile phase is flowing through the system. The M⁺ mass of your derivatized analyte should be Analyte mass+332.17 Da.

8. Look for the Q1 mass and obtain the accurate mass of the derivative, which is analyte mass+332.17, and then optimize DP and EP parameters. If necessary, dilute the sample so that the intensity does not exceed 2×10^6 counts.
9. Switch to Product Ion Scan method and optimize the CE value in which most specific and intense fragments are achieved (ramp the CE parameter)
10. Switch to MRM method, enter the MRM transitions obtained from previous step and optimize the rest of the parameters for highest intensity (CXP, TEM, IS, Gas, etc.)

Note: *Labeling can provide one or more isomeric product peaks depending on the molecule being labeled.*

Sample Preparation

1. Prepare biological sample according to your laboratory optimized protocol.
 - Commonly used methods are solid phase extraction (SPE), protein precipitation and liquid-liquid extraction (LLE).
2. Dry the matrix obtained after sample preparation in a microcentrifuge tube.
 - Commonly used methods are speed vacuum evaporation or drying using a stream of nitrogen.



Amplifex™ Diene Reagent Solution Preparation and Labeling Protocol

1. Transfer (2.5 mL) of the Amplifex™ Diene Diluent to the tube of Amplifex™ Diene Reagent.
2. Vortex the mixture. If necessary, sonicate briefly (~30 sec) to help the solid dissolve completely.
3. Add 50 µL of the reagent solution to the dried sample prepared above, cap and vortex for 30 seconds and wait for 30 min. at ambient temperature.
4. Add 50 µL of deionized water and mix.

Note: *Amplifex™ Diene-reagent solution should be used within 2 hours of dilution. The reagent will be deactivated in the presence of protic solvents, avoid exposure of the reagent solution to excess moisture.*

LC/MS Analysis

1. Use optimized LC/MS method as developed above to analyze the sample.

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