

Amplifex™ Diene Cartridge Kit

Sample preparation for the analysis of 1,25-Dihydroxy Vitamin D3 (1,25-DHVD3) and 1,25-Dihydroxy Vitamin D2 (1,25-DHVD2) in serum using the solid liquid extraction single use cartridges.

Sample Volume: 200 µL plasma or serum

A. Equipment and Supplies

- Amplifex™ Diene Cartridge Kit PN 4466052
- Cartridge Rack PN 4466059
- 0.5 mL Eppendorf Safe-Lock microcentrifuge Tubes (product number 022363611)
- 2 mL Eppendorf Safe-Lock microcentrifuge Tubes (product number 022363352)
- 5 mL polypropylene tubes (with conical bottom and free standing base, VWR Cat. # 16465-262)
- Disposable flint glass culture tubes, 18 x 150 mm (VWR Cat. #60825-443)
- Vortex mixer
- Microcentrifuge
- 5 mL, 1 mL, 200 µL and 20 µL pipettes
- Pipette tips (RT 1000F, RT 200F, and RT 20F tips from Rainin are recommended to avoid cross contamination)
- Gloves

B. User Provided Chemicals

- Di-isopropyl ether (Aldrich product number 38279)
- De-ionized water (MilliQ, 18 MΩ)
- iso-Propanol (HPLC Grade, Aldrich, product number 278475)
- Hexanes (HPLC or GC Grade, JT BAKER, product number 9304-03)
- Methanol (HPLC Grade, ACROS, AC61009-0040)
- Calibration standards and internal standards as necessary
- Calibration matrix (recommend using matrices similar to the sample matrix and free of analytes)

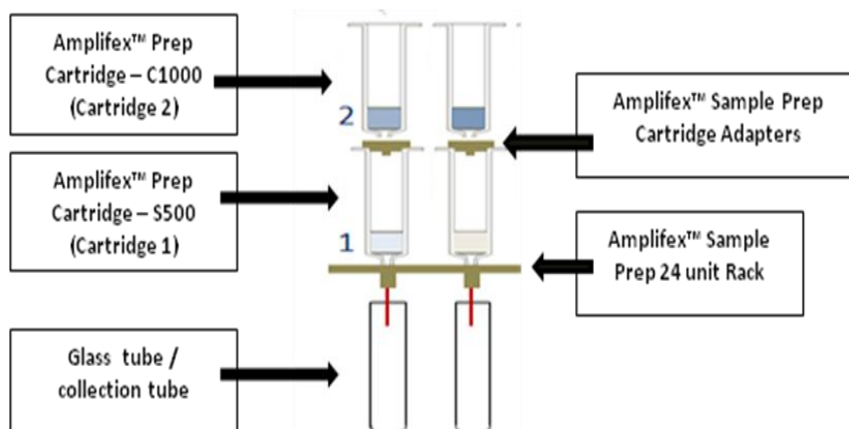
C. Sample Preparation



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.

Tip: The analytes are light sensitive. Do not expose standards and samples to strong lighting.

- Solvent preparation:
 - **Wash solvent 1:** 4% (v/v) iso-Propanol in hexanes (usage: 9 mL/sample)
 - **Wash solvent 2:** 6% (v/v) iso-Propanol in hexanes (usage: 6 mL/sample)
 - **Elution solvent:** 30% (v/v) iso-Propanol in hexanes (usage: 4.5 mL/sample)
- Equilibrate all samples, standards, internal standards (**IS**), calibration matrices (**Cal. Matrix**), and other chemicals to ambient temperature (22–25 °C).
- For samples and controls, dispense 200 µL serum samples into a 2 mL micro centrifuge tubes. Dilute the samples with 500 µL of deionized water, mix gently (**avoid foaming**) and thoroughly by pulse vortex and spin 30 seconds using a microcentrifuge.
- For calibration standard curve, prepare standard mixture solutions and internal standard mixture solutions of appropriate concentrations. Prepare calibration matrix accordingly (e.g., 200 µL serum matrix plus 500 µL of deionized water for each calibration concentration point).
- Assemble Cartridges as illustrated below. Place glass tubes under cartridge 1 to collect washing flow through. **Label the cartridges with sample ID as necessary.**



- For each sample (unknowns, standards and controls), Spike 20 µL of the internal standard mixture solution (**prepared in step 4**) on top of the cartridge 2.
- For calibration curve, spike 20 µL of each calibration standard mix solution (**step 4**) on top of the cartridge 2 respectively. Then load 700 µL of the diluted Cal. Matrix (**step 4**) on top of each cartridge 2 respectively. Wait for 10 minutes.
 - For samples and controls, load 700 uL of the diluted serum samples (**step 3**) on top of the cartridge 2 respectively. Wait for 10 minutes.

8. Elute analytes from cartridge 2 to cartridge 1 with 4 times 1 mL di-isopropyl ether. Wait 3 minutes between each elution.
9. Remove the adapters and cartridge 2s. Leave the adapters and cartridge 2s in a well ventilated hood overnight. Dispose cartridge 2s properly. The adapters can be reused after rinsing with acetone (ACS grade or better) and deionized water, dry completely before using.
10. Wash Cartridge 1 with 4% iso-propanol/hexanes 2 times of 4.5 mL. Do not use vacuum suction during the washings.
11. Wash Cartridge 1 with 6% iso-propanol/hexanes 1 time of 6 mL. Do not use vacuum suction during the washings.
12. Discard the washes and glass tubes properly.
13. Adjust the rack height and place **labeled** 5 mL collection tubes under the respective cartridges.
14. Elute the analytes with 4.5 mL of 30% iso-propanol/hexanes into the 5 mL collection tubes.
15. Dry the samples in the collection tube (to complete dryness) using a speed vacuum concentrator at ambient temperature.
Tip: Cover the top of the speed vac. with aluminum foil if necessary to keep the samples out of light exposure during this process.
16. Add 300 μ L of methanol in each 5 mL tube, vortex for 1 minute, spin down, then transfer the content to a **labeled** 0.5 mL Eppendorf Safe-Lock microcentrifuge tube and dry the samples again in a speed vac. at ambient temperature.
17. Follow Amplifex™ Diene Reagent Protocol for derivatization or reconstitute in appropriate LC/ MS solvent.

D. Table – Part Information

Part number	Description
4466053	Amplifex™ Diene Cartridge Prep Starter Kit
	Amplifex™ Prep – C1000 24 pack
	Amplifex™ Prep – S500 24 pack
	Amplifex™ Sample Prep Cartridge Adapters
	Amplifex™ Sample Prep 24 unit Rack
4466052	Amplifex™ Diene Cartridge Kit
	Amplifex™ Prep – C1000 24 pack
	Amplifex™ Prep – S500 24 pack
4466059	Amplifex™ Sample Prep 24 unit Rack
4465966	Amplifex™ Diene Kit



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Part Number 4466056 Rev. A
3/2013