analysis of phospholipids from sediment samples

chemicals:

chloroform (pure)
methanol (pure)
phosphate buffer:
8.7 g K_2HPO_4 filled up with Aqua dest. to a final volume of 1 liter; pH 7.4
potassium-peroxodisulfate-solution:
add 5 g $K_2S_2O_8$ to 100 ml 0.36N H_2SO_4
ammonium-molybdat-solution:
add 2.5 g $(NH_4)_6$ Mo $_7$ O_{24} A 4 H2O to 97.5 ml 5.97N H_2SO_4
malachit-green-solution:
add 0.113 g Polyvinylalcohol (98%) to 100 ml of warm (80°C) Aqua. dest.;
let it cool down and add 0.011 g malachit-green

accessories:

50 ml Oak Ridge teflon centrifugation vials (solvent resistant) with screw-caps;
centrifuge;
multi-varipettes (50, 12.5, 2.5 ml);
Eppendorf-varipettes (10-100 Ìl, 100-1000 Ìl);
vacuum pump;
glass funnels and Whatman-filters (2 V, 12.5 cm);
Wheaton vials (2 ml);
welding torch for glass vials;
nitrogen gas cylinder.

method:

first day:

1.) add 2 ml of wet sediments to the centrifuge test tubes
2.) add 1 ml phosphate buffer, 4 ml chloroform and 8 ml methanol
3.) block up the tubes and give it a good shake (homogenized mixture)
4.) leave it for the next day

second day:

1.) add 4 ml Aqua. dest. and 4 ml chloroform (seperation into two phases)
2.) block up the tubes and give it a good shake (homogenized mixture)
3.) leave it for the next day

third day:

1.) centrifugation: 10 min. at 6000 rpm, 400 x g
2.) suck of the water phase quantitatively
3.) filter the chlorophyll phase into glass test tubes
4.) pipette 2 ml of the chloroform extract into a glass vial
5.) evaporate the chloroform with nitrogen in a water bath at 40°C
6.) when vials are totally dry inside add 0.5 ml potassium-peroxodisulfat-solution
7.) heat seal the glass vials
8.) incubate vials at 95°C till next day

forth day:

1.) open vials and
2.) add 0.1 ml ammonium-molybdat-solution and leave it for 10 min.
3.) add 0.5 ml malachit-green-solution and leave it for 30 min.
4.) measurement of the final solution at 610 nm with a photometer

standards

0.6805 g K_2HPO_4 filled up with Aqua dest. to a final volume of 100 ml;
1 ml of this solution filled up with Aqua. dest. to a final volume of 100 ml
= standard solution of 0.5 Ìmol/ml.

ml Aqua. dest.	ml standard solution	nmol/ml end concentration
1.00	0.00	0.0
0.95	0.05	0.5
0.90	0.10	1.0
0.80	0.20	2.0
0.60	0.40	4.0
0.40	0.60	6.0
0.00	1.00	10.0

Add 20 Ìl of each end conc. solution to 0.5 ml potassium-peroxodisulfat for measurements.

Literature:

Findlay, R.H., G.M. King & L. Watling (1989): Efficiancy of phospholipid analysis in determining microbial biomass in sediments. - Appl. Environ. Microbiol., **55**: 2888-2893.