# Core K: Human plasma SRM neutral folch extraction and lipid analysis

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**Extraction Materials:**

Chloroform HPLC grade (Mallinckrodt Baker)
Methanol HPLC grade (Mallinckrodt Baker)
Sterile phosphate buffered saline
Dimethyl sulfoxide (DMSO)
Disposable Pyrex screw cap glass tubes (Corning) with Teflon coated tops
Pasteur pipettes (Fisher)
Glass Target DP vial for liquid chromatography (National Scientific)
Cardiolipin internal standard mixture 1 (Avanti Polar Lipids)
(LIPID MAPS ID: LMGP12010002-LMGP12010005
Coenzyme $Q_6$ from *Saccharomyces cervisiae* (Sigma)
Nor-dolichol-[13-22] (Avanti Polar Lipids)
LIPID MAPS ID: LMPR03070023
1,2-dioleoyl-*sn*-glycero-3-phospo-L-serine-*N*-nonadecanoyl (Avanti Polar Lipids)

**Standard Solution Preparation:**

1. Dilute 1,2-dioleoyl-*sn*-glycero-3-phospo-L-serine-*N*-nonadecanoyl standard in $CHCl_3$ to final concentration of 20 ng/µL.
2. Combine 1 mL coenzyme $Q_6$ (0.1 mg/mL), 900 µL cardiolipin internal standard mixture 1 (10 µM each standard), and 100 µL nor-dolichol-[13-22] (0.1 mg/mL) in a disposable glass tube with fresh Teflon lined cap. Vortex until the standard solution is uniform. The final concentrations of coenzyme $Q_6$, cardiolipin internal standard mixture 1, and nor-dolichol-[13-22] will be 0.05 mg/mL, 4.5 µM, and 5 µg/ml, respectively.

**Neutral Folch [(1)] Extraction:**

1. Remove human plasma SRM aliquot from -80 °C freezer and thaw at room temperature.
2. Combine 4 mL $CHCl_3$, 2 mL methanol, and 1.17 mL PBS in disposable glass tube with fresh Teflon lined cap. Add to this 10 µL of the prepared standards mix and 2 µL of the diluted 1,2-dioleoyl-*sn*-glycero-3-phospo-L-serine-*N*-nonadecanoyl. Finally, add 0.33 mL of the thawed human plasma SRM.
3. Vortex samples until they are uniform in color and cell density.
4. Let samples incubate at room temperature for 30 minutes on rotary shaker.
5. Spin in table top centrifuge for 20 minutes at 2500 x g.
6. Transfer organic lower phase to a fresh disposable glass tube and dry under a stream of $N_2$ gas.
7. Resuspend dried lipid with approximately 1.5 mL $CHCl_3$, sonicate, and transfer to a glass LC vial.

8. Dry resuspended extract under a stream of $N_2$ gas. Store dried lipid at -20 °C.

**Mass spectrometry sample preparation:**

1. Remove samples from -20 °C freezer and thaw at room temperature.
2. Resuspend sample in 100 µL of $CHCl_3$, vortex, and sonicate.
3. For cardiolipin analysis on Q-Star, draw 10 µL of resuspended lipid in $CHCl_3$ and dilute 1:10 (*v:v*) in a fresh LC vial.
4. For dolichol and coenzyme Q analysis on 4000 Q-Trap, draw 20 µL of resuspended lipid in $CHCl_3$ and transfer to a fresh LC vial. Dry lipid under a stream of $N_2$ gas and resuspended in 100 µL methanol/DMSO (1:1, *v:v*).
5. For *N*-acyl-PS analysis on 4000 Q-Trap, draw 50 µL of resuspended lipid in $CHCl_3$ and dilute 1:1(*v:v*) in a fresh LC vial.

**Cardiolipin liquid chromatography-mass spectrometry:**

The analysis was performed with normal-phase liquid chromatography coupled with tandem mass-spectrometry. The normal-phase liquid chromatography procedure was described previously [(2)], with modifications made to the elution program. Briefly, the elution program consisted of the following: 100% mobile phase A was held isocratically for 2 min and then linearly increased to 100% mobile phase B over 14 min and held at 100% B for 11 min. The LC gradient was then changed to 100% mobile phase C over 3 min and held at 100% C for 3 min, and finally returned to 100% A over 0.5 min and held at 100% A for 5 min. The total LC flow rate was 300 µl/min. An injection volume of 30 µL of the diluted sampled was generally sufficient. Cardiolipin internal standard mixture 1 (Avanti Polar Lipids) was used as the internal standard to determine the limit of detection. Data acquisition and analysis were performed using Analyst QS software (Applied Biosystems).

**Dolichol and ubiquinone analysis**

The analysis was performed with reverse-phase chromatography coupled with MRM, as described [(3)]. An injection volume of 15 µL of the diluted sampled was generally sufficient. For dolichol analysis, samples were run in the negative ion mode and were monitored for singly-charged dolichol-acetate adduct ions $[M+CH_3CO_2]^-$ paired with the loss of the acetate ion (59.000 amu) for each analyte and nor-dolichol standard. For coenzyme Q analysis, samples were run in the positive ion mode and were monitored for singly-charged coenzyme Q-ammonium adduct ions $[M+NH_4]^+$ paired with the major fragment ion corresponding to a proton adduct of the quinone ring of coenzyme Q (197.000 amu) for each analyte and the internal standard. Nor-dolichol-[13-22] (Avanti Polar Lipids) and yeast coenzyme $Q_6$ (Sigma) were used as internal standards for quantitation. Data acquisition and analysis were performed using Analyst 1.4 software (Applied Biosystems).

**Table 1**: Ubiquinone negative-mode MRM pairs

| Analyte | $Q_1$ Mass (amu) | $Q_3$ Mass (amu) |
|---|---|---|
| Yeast Coenzyme $Q_6$ | 608.500 | 197.000 |
| Coenzyme $Q_9$ | 812.700 | 197.000 |
| Coenzyme $Q_{10}$ | 880.700 | 197.000 |

**Table 2**: Dolichol negative-mode MRM pairs

| Analyte | $Q_1$ Mass (amu) | $Q_3$ Mass (amu) |
|---|---|---|
| Dolichol 16 | 1168.500 | 59.000 |
| Dolichol 17 | 1236.500 | 59.000 |
| Dolichol 18 | 1304.500 | 59.000 |
| Dolichol 19 | 1372.500 | 59.000 |
| Dolichol 20 | 1440.500 | 59.000 |
| Dolichol 21 | 1508.500 | 59.000 |
| Nor-dolichol 16 | 1168.500 | 59.000 |
| Nor-dolichol 17 | 1222.500 | 59.000 |
| Nor-dolichol 18 | 1290.500 | 59.000 |
| Nor-dolichol 19 | 1358.500 | 59.000 |
| Nor-dolichol 20 | 1426.500 | 59.000 |
| Nor-dolichol 21 | 1494.500 | 59.000 |

## *N*-acyl-PS analysis

The analysis was performed with normal-phase liquid chromatography, described above, coupled with multiple reaction monitoring (MRM), as described [3]. Synthetic 1,2-dioleoyl-*sn*-glycero-3-phospho-L -serine-*N*-nonadecanoyl (Avanti Polar Lipids) was used as an internal standard for quantitation. Data acquisition and analysis were performed using Analyst 1.4 software (Applied Biosystems).

**Table 3**: *N*-acyl-PS negative mode MRM pairs

| Analyte | $Q_1$ Mass (amu) | $Q_3$ Mass (amu) |
|---|---|---|
| 58:1 | 1026.800 | 701.500 |
| 60:2 | 1052.800 | 701.500 |
| Internal Standard | 1066.900 | 699.600 |

## References


1. Folch J, Lees M, Sloane Stanely GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957;226:497-509.

2. Garrett TA, Raetz CR, Richardson T, Kordestani R, Son JD, Rose RL. Identification of phosphatidylserylglutamate: a novel minor lipid in *Escherichia coli*. *J Lipid Research.* 2009;50:1589-1599.

3. Guan Z, Li S, Smith D, Shaw W, Raetz CRH. Identification of *N*-acylphosphatidylserine molecules in eukaryotic cells. *Biochemistry*. 2007;46:145000-14513.