Preparing Test Samples / TOF G2-S/XS Instruments

This procedure describes how to prepare test stock solutions and diluted working solutions when required, from the original compounds supplied in the TOF G2-S/XS sample kits (700008842, 700008892, 700008893).

Parts required

Table 1: TOF G2-S/XS sample kits

Part number	Description	Qty
700008842	TOF G2-S Sample Kit - 1	1
700008892	TOF G2-S Sample Kit - 2	1
700008893	TOF G2-S Sample Kit - 3	1

Table 2: Sample kit 700008842 components

Serial	Sample	Supply format	Amount required in each vial	Number of vials
1	Leucine Enkephalin	Powder	3 ±0.06 mg	2
2	[Glu^1]-fibrinopeptide B	Powder	0.1 ±0.01 mg	2
3	MassPREP™ Digestion Standard, Alcohol Dehydrogenase	Powder	1 nmol	1
4	MassPREP™ Digestion Standard, Enolase	Powder	1 nmol	1

Table 3: Sample kit 700008892 components

Serial	Sample	Supply format	Amount required in each vial	Number of vials
1	Sodium Iodide	Solution	25 mL	1
5	Sodium Hydroxide	Solution	25 mL	1
3	PEG MALDI	Solution	2 mL	1
4	6 Mix Solution	Solution	-	1
5	Chloramphenicol	Powder	50 mg	1
6	Sulfadimethoxine	Powder	5 mg	1

Table 4: Sample kit 700008893 components

Serial	Sample	Supply format	Amount required in each vial	Number of vials
1	D(+)-Raffinose	Powder	2.5 ±0.05 mg	2
2	Matrix	Powder	10 ±0.2 mg	5
3	Formic Acid	Solution	1 mL	2
4	Aqueous Ammonia	Solution	3 mL	1

Tools/materials required

Some or all of the following may be required during sample preparation.

Solvents

- LCMS-grade acetonitrile
- LCMS-grade methanol
- HPLC-grade 2-propanol
- LCMS-grade deionized water

NOTE: *Use solvents of the appropriate grade; Waters recommends Fisher Scientific high purity solvents.*

Additives

- >99.5% purity Glacial acetic acid
- >98% purity formic acid, supplied within Kit-3, if required a 10 mL bottle may be ordered using 700002341
- Ammonia solution (specific gravity 0.88 to 0.89)
- ≥99% HPLC-grade trifluoroacetic acid

NOTE: *Waters recommends Sigma Aldrich and BDH additive suppliers (Aristar grade), and to avoid using GPR grade additives.*

Containers

- 1.5-mL disposable plastic vial
- 5-mL disposable glass vials (with caps)
- 10-mL graduated measuring cylinder
- 100-mL graduated measuring cylinder
- 10-mL volumetric flask
- 20-mL volumetric flask
- 100-mL Duran bottle
- 30-mL Nalgene fluidics container (with caps) - provided with instrument
- 2-mL ACQUITY vials

NOTE: *All glassware must be clean, rinsed with methanol, and dry prior to use. Do not use any detergents.*

Pipettes (and tips)

- 1-µL pipette
- 20-µL pipette
- 200-µL pipette
- 1000-µL pipette
- 5-mL pipette

NOTE: *All pipettes used to make up samples must be calibrated and within specification. Use appropriate pipettes and tips whenever possible during the sample dilution steps (see Appendix for pipette volume ranges).*

Procedure

WARNING: ***Health and Safety policies of Waters and the laboratory in use must be adhered to.***

NOTE: *Take care when opening the vials not to spill the sample. Samples prepared in glass volumetric flasks must be aliquoted into suitable containers (Nalgene or PTFE) before storing in a freezer.*

1. Sonicate samples to ensure they dissolve completely.
2. Label all samples with the following details before being stored:
   - Sample name
   - Concentration and composition of solvent
   - Preparation date
   - Expiry date

NOTE: *Prepare all solvents and solvent mixtures quantitatively. Allow liquid samples to warm to room temperature before use.*

Diluent preparation

50:50 acetonitrile/water

1. Using a 100-mL measuring cylinder, transfer 30 mL LCMS grade acetonitrile to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 30 mL deionized or LCMS grade water to the 100-mL Duran bottle.
3. Sonicate for 10 minutes and label as "50:50 acetonitrile/water".

50:50 acetonitrile/water + 0.1% formic acid

1. Using a 100-mL measuring cylinder, transfer 50 mL LCMS grade acetonitrile to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 50 mL deionized or LCMS grade water to the 100-mL Duran bottle.
3. Using a 200-µL pipette, add 100 µL formic acid to the 100-mL Duran bottle.
4. Sonicate for 10 minutes and label as "50:50 acetonitrile/water + 0.1% formic acid".

50:50 methanol/water + 0.1% formic acid (nanoACQUITY systems only)

1. Using a 100-mL measuring cylinder, transfer 50 mL LCMS grade methanol to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 50 mL deionized or LCMS grade water to the 100-mL Duran bottle.
3. Using a 200-µL pipette, add 100 µL formic acid to the 100-mL Duran bottle.
4. Sonicate for 10 minutes and label as "50:50 methanol/water + 0.1% formic acid".

20:80 acetonitrile/water + 0.1% formic acid (nanoACQUITY systems only)

1. Using a 10-mL measuring cylinder, transfer 10 mL LCMS grade acetonitrile to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 40 mL deionized or LCMS grade water to the 100-mL Duran bottle.
3. Using a 200-µL pipette, add 50 µL formic acid to the 100-mL Duran bottle.
4. Sonicate for 10 minutes and label as "20:80 acetonitrile/water + 0.1% formic acid".

5:95 acetonitrile/water + 0.1% formic acid (nanoACQUITY systems only)

1. Using a 1000-µL pipette, transfer 250 µL LCMS grade acetonitrile to a 5-mL glass vial.
2. Using a 5-mL pipette, transfer 4750 µL deionized or LCMS grade water to the 5-mL glass vial.
3. Using a 1-µL pipette, add 0.5 µL formic acid to the 5-mL glass vial.
4. Sonicate for 10 minutes and label as "5:95 acetonitrile/water + 0.1% formic acid".

Water + 0.01% formic acid + 0.05% ammonia solution (pH9) (ACQUITY systems only)

1. Measure 500 mL of filtered LCMS-grade water and then transfer to a 500-mL reservoir bottle.
2. Using a 200-µL pipette, add 50 µL of formic acid to the 500-mL reservoir bottle.
3. Using a 1000-µL pipette, add 250 µL of ammonia solution to the 500-mL reservoir bottle.
4. Recap, shake well, and label as "LCMS diluent".

Trifluoroacetic acid 0.1% aqueous (MALDI only)

1. Using a 100-mL measuring cylinder, transfer 50 mL deionized or LCMS grade water to a 100-mL Duran bottle.
2. Using a 100-µL pipette, add 50 µL TFA to the 100-mL Duran bottle.
3. Sonicate for 10 minutes and label as "TFA 0.1% aqueous".

50:50 TFA 0.1% aqueous/acetonitrile (MALDI only)

1. Using a 100-mL measuring cylinder, transfer 25 mL "TFA 0.1% aqueous" to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 25 mL deionized or LCMS grade acetonitrile to the 100-mL Duran bottle.
3. Sonicate for 10 minutes and label as "50:50 acetonitrile/water + 0.1% TFA".

Sample preparation (700008842)

Leucine enkephalin

- $C_{28}H_{37}N_5O_7$
- Tyr Gly Gly Phe Leu
- (M+H) *m/z* 556.2771, (M-H) *m/z* 554.2615 (MassLynx masses)
- $(M+H)^+$ *m/z* 556.2766, $(M-H)^-$ *m/z* 554.2620 (UNIFI masses)

1 ng/µL leucine enkephalin

1. Using a 5-mL pipette, add 7.5 mL of water to the contents of a 3 mg bottle of leucine enkephalin.
2. Recap, shake well, and sonicate for five minutes.
3. Label as "400 ng/µL leucine enkephalin in water" and store in the freezer (expires in three months).
4. Using a 200-µL pipette, transfer 50 µL of the 400 ng/µL leucine enkephalin in water to a 20-mL volumetric flask.
5. Make up to 20 mL with 50:50 acetonitrile/water + 0.1% formic acid and sonicate for five minutes.
6. Transfer from the 20-mL volumetric flask to a 30-mL fluidics container.
7. Label as "1 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the freezer (expires in one month).

200 pg/µL leucine enkephalin

1. Using a 1000-µL pipette, transfer 4000 µL of the 1 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 acetonitrile/water + 0.1% formic acid and sonicate for five minutes.
3. Label as "200 pg/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the refrigerator (expires in one month).

50 pg/µL leucine enkephalin

1. Using a 1000-µL pipette, transfer 1000 µL of the 1 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 acetonitrile/water + 0.1% formic acid and sonicate for five minutes.
3. Label as "50 pg/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the refrigerator (expires in one week).

[Glu^1]-fibrinopeptide B (GluFib)

- $C_{66}H_{95}N_{19}O_{26}$
- Glu Gly Val Asn Asp Asn Glu Glu Gly Phe Phe Ser Ala Arg
- (M+H) *m/z* 1570.6774, (M+2H) *m/z* 785.8426, (M-H) *m/z* 1568.6617, $(M-2H)^{2-}$ *m/z* 783.8270 (MassLynx masses)
- $(M+H)^{+}$ *m/z* 1570.6768, $(M+2H)^{2+}$ *m/z* 785.8421, $(M-H)^{-}$ *m/z* 1568.6623, $(M-2H)^{2-}$ *m/z* 783.8275 (UNIFI masses)

NOTE: *Many proteins are "sticky" by nature, so to avoid material adhering to the outside of the pipette, do not immerse the pipette tip any further than necessary to retrieve a sample.*

ESI

1 pmol/µL GluFib solution

1. Using a 1000-µL pipette, add 2000 µL of deionized or LCMS-grade water to the contents of a 0.1 mg bottle of GluFib and sonicate for five minutes.
2. Label as "32 pmol/µL GluFib stock solution" and store in the freezer (expires in three months).
3. Using a 200-µL pipette, transfer 100 µL of the 32 pmol/µL GluFib stock solution to a 5-mL glass vial.
4. Using a 5-mL pipette, add 3 mL of 50:50 acetonitrile/water + 0.1% formic acid and using a 200-µL pipette, add 100 µL of 50:50 acetonitrile/water + 0.1% formic acid to the 5-mL glass vial.
5. Sonicate for five minutes and label as "1 pmol/µL GluFib solution 50:50 acetonitrile/water + 0.1% formic acid". Store in the freezer (expires in one week).

100 fmol/µL GluFib solution

1. Using a 1000-µL pipette, transfer 2000 µL of the 1 pmol/µL GluFib solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 acetonitrile/water + 0.1% formic acid and sonicate for five minutes.
3. Transfer from the 20-mL volumetric flask to a 30-mL fluidics container.
4. Label as "100 fmol/µL GluFib solution in 50:50 acetonitrile/water + 0.1% formic acid" and store in the freezer (expires in one week).

Nano ESI systems

100 fmol/µL GluFib solution (for analyte sprayer setup)

1. Using a 1000-µL pipette, transfer 2000 µL of the 1 pmol/µL GluFib solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 20:80 acetonitrile/water + 0.1% formic acid and sonicate for five minutes.
3. Transfer from the 20-mL volumetric flask to a 30-mL fluidics container.
4. Label as "100 fmol/µL GluFib solution in 20:80 acetonitrile/water + 0.1% formic acid" and store in the freezer (expires in one week).

100 fmol/µL GluFib solution (for reference sprayer)

1. Using a 1000-µL pipette, transfer 312.5 µL of the 32 pmol/µL GluFib solution to a 100-mL volumetric flask.
2. Make up to 100 mL with 50:50 methanol/water + 0.1% formic acid and sonicate for five minutes.
3. Transfer from the 100-mL volumetric flask to a suitable container for storage on top of the nanoACQUITY and label as "100 fmol/µL GluFib solution in 50:50 methanol/water + 0.1% formic acid".

MALDI

NOTE: *Use the same 32 pmol/µL GluFib stock solution as for ESI.*

200 fmol/µL GluFib solution

1. Using a 200-µL pipette, transfer 100 µL of the 32 pmol/µL GluFib stock solution to a 5-mL glass vial.
2. Using a 5-mL pipette, add 3 mL of 0.1% TFA aqueous and using a 200-µL pipette, add 100 µL of 0.1% TFA aqueous to the 5-mL glass vial.
3. Sonicate for five minutes and label as "1 pmol/µL GluFib solution in 0.1% TFA". Store in the freezer (expires in one week).
4. Using a 200-µL pipette, transfer 200 µL of 1 pmol/µL GluFib solution to a 1.5-mL plastic vial.
5. Using a 1000-µL pipette, add 800 µL of 0.1% TFA aqueous and sonicate for five minutes.
6. Label as "200 fmol/µL GluFib solution in 0.1% TFA" and store in the freezer (expires in one week).

20 fmol/µL GluFib solution

1. Using a 200-µL pipette, transfer 100 µL of 1 pmol/µL GluFib solution to a 5-mL glass vial.
2. Using a 5-mL pipette, add 4.9 mL of 0.1% TFA aqueous and sonicate for five minutes.
3. Label as "20 fmol/µL GluFib solution in 0.1% TFA" and store in the freezer (expires in one week).

100 fmol/µL and 10 fmol/µL GluFib MALDI solution

1. Dilute the 200 fmol/µL GluFib solution with 3.6 mg/mL matrix to a 1:1 ratio, in a 1.5-mL plastic vial, to create 100 fmol/µL.
2. Dilute the 20 fmol/µL GluFib solution with 3.6 mg/mL matrix to a 1:1 ratio, in a 1.5-mL plastic vial, to create 10 fmol/µL.
3. Using a 1-µL pipette, spot 1 µL of the GluFib and matrix mixture onto the sample plate wells.

   NOTE: *These dilutions expire the following day and must be used immediately.*

MassPREP™ Digestion Standard, alcohol dehydrogenase

(Field Service Engineer to prepare)

1. Add 100 µL of 5:95 acetonitrile/water + 0.1% formic acid to the 1-nmol vial of ADH and label as "10 pmol ADH stock solution" (expires in one month).

MassPREP™ Digestion Standard, enolase

(Field Service Engineer to prepare)

1. Add 100 µL of 5:95 acetonitrile/water + 0.1% formic acid to the 1-nmol vial of enolase and label as "10 pmol enolase stock solution" (expires in one month).

MassPREP™ Digestion Standard, alcohol dehydrogenase and enolase mix

1. Using a 20-µL pipette, add 10 µL of enolase stock solution to a 1.5-mL disposable glass vial.
2. Using a 20-µL pipette, add 10 µL of ADH stock solution to the same vial.
3. Add 980 µL 5:95 acetonitrile/water + 0.1% formic acid to the same vial, to create 100 fmol/µL enolase + ADH mix stock solution, recap the vial and sonicate for five minutes.
4. Label as "100 fmol/µL enolase + ADH mixture stock solution".
5. Using a 100-µL pipette, add 25 µL of 100 fmol/µL enolase + ADH stock solution to a Waters maximum recovery vial.
6. Add 75 µL 5:95 acetonitrile/water + 0.1% formic acid to the same vial, to create 25 fmol/µL enolase + ADH mixture test solution, recap the vial and sonicate for five minutes.
7. Label as "25 fmol/µL enolase + ADH mixture test solution".

Sample preparation (700008892)

Sodium iodide

(Vendor prepared, use as provided)

1. Dissolve 50 ±1.0 mg of sodium iodide in 25 mL of 50:50 2-propanol/water and sonicate for five minutes.
2. Label as "2 µg/µL sodium iodide solution", this can be stored on the bench (expires 12 months after opening).

Sodium hydroxide to make "sodium formate"

(Vendor prepared, use as provided)

- 0.5 mM sodium hydroxide in 90:10 2-propanol/water

(Field Service Engineer to prepare)

0.5 mM sodium formate solution in 90:10 2-propanol/water

1. Using a 1000-µL pipette, transfer 900 µL of deionized or LCMS-grade water to the 5-mL vial.
2. Using a 200-µL pipette, add 100 µL formic acid to the 5-mL vial and shake well.
3. Using a 200-µL pipette, transfer 100 µL of the above 10% formic/aqueous solution to the 30-mL nalgene bottle containing the 0.5mM sodium hydroxide and sonicate for five minutes.
4. Label as “0.5 mM sodium formate”, store in the refrigerator (expires in three months).

PEG MALDI

(Vendor prepared, use as provided)

NOTE: *Individual PEG 600, 1000, 1500, and 2000 are NOT supplied in the kit (for reference only).*

1. Dilute 10 mg of PEG 600 with 1000 µL of 50:50 water/acetonitrile, to create a 10 mg/mL solution of PEG 600.
2. Dilute 10 mg of PEG 1000 with 1000 µL of 50:50 water/acetonitrile, to create a 10 mg/mL solution of PEG 1000.
3. Dilute 10 mg of PEG 1500 with 1000 µL of 50:50 water/acetonitrile, to create a 10 mg/mL solution of PEG 1500.
4. Dilute 10 mg of PEG 2000 with 1000 µL of 50:50 water/acetonitrile, to create a 10 mg/mL solution of PEG 2000.
5. Mix 500 µL of each PEG solution 600, 1000, 1500, and 2000 together.
6. Sonicate for five minutes and label as "PEG MALDI solution”. This can be stored on the bench (expires 12 months after opening).

Specification solution

(Field Service Engineer to prepare)

1. Using a 1000-µL pipette, transfer 1000 µL of the PEG MALDI solution to a 1.5-mL plastic vial.
2. Using a 200-µL pipette, add 150 µL of 2 µg/µL sodium iodide solution to the 1.5-mL plastic vial (refer to "Sodium iodide" section) and sonicate for five minutes.
3. Label as "PEG MALDI specification solution (with sodium iodide)" and store in the refrigerator (expires in one week).
4. Using a 1.5-mL plastic vial, dilute the PEG MALDI solution (with sodium iodide) with 3.6 mg/mL matrix to a 1:1 ratio, to create the PEG MALDI sample.
5. Using a 1-µL pipette, spot 1 µL of the PEG and matrix mixture onto the sample plate wells.

NOTE: *This sample expires the following day and must be used immediately.*

6 Mix solution

(Field Service Engineer to prepare)

The 6 mix solution contains the components listed in Table 5:

Table 5: 6 Mix solution components and expected masses

Component	Sample Concentration	Expected Molecular Mass observed in MassLynx		Expected Ionic Mass observed in UNIFI	
Acetaminophen - $C_8H_9NO_2$	2 ng/µL	[M+H] 152.0712	N/A	$[M+H]^+$ 152.0706	N/A
Caffeine - $C_8H_{10}N_4O_2$	2 ng/µL	[M+H] 195.0882	N/A	$[M+H]^+$ 195.0877	N/A
Sulfadimethoxine - $C_{12}H_{14}N_4O_4S$	1 ng/µL	[M+H] 311.0814	[M-H] 309.0658	$[M+H]^+$ 311.0809	$[M-H]^-$ 309.0663
Verapamil - $C_{27}H_{38}N_2O_4$	500 pg/µL	[M+H] 455.2910	N/A	$[M+H]^+$ 455.2904	N/A
Chloramphenicol - $C_{11}H_{12}Cl_2N_2O_5$	500 pg/µL	N/A	[M-H] 321.0045	N/A	$[M-H]^-$ 321.0051
17-α-hydroxyprogesterone – $C_{21}H_{30}O_3$	50 ng/µL	[M+H] 331.2273	N/A	$[M+H]^+$ 331.2268	N/A

NOTE: *Before opening the ampoule containing "TOF G2-S 6 Mix solution", sonicate for three minutes, and then vortex mix the ampoule. Doing so ensures that the solution is homogenous before sampling.*

Serial dilutions of the 6 Mix stock solution is required to obtain the relevant target concentrations required for the instrument specific LCMS system installation tests.

SYNAPT G2-S/Si

6 Mix tuning solution

1. Using a 1000-µL pipette, add 1000 µL of 6 Mix stock solution to a 10-mL volumetric flask.
2. Make up to 10 mL with LCMS diluent and sonicate for five minutes.
3. Label as "6 Mix diluted" and store in the refrigerator (expires in one month).

6 Mix solution - 10 pg/µL of sulfadimethoxine and 5 pg/µL chloramphenicol

1. Using a 200-µL pipette, add 100 µL of the 6 Mix tuning solution to a 2-mL ACQUITY vial.
2. Using a 1000-µL pipette add 900 µL of LCMS diluent, recap and vortex the vial to mix fully.
3. Label the vial "6 Mix 10 pg/µL SDM" and store in the refrigerator (expires in one week).

Chloramphenicol

- $C_{11}H_{12}N_2Cl_2O_5$
- (M-H) *m/z* 321.0045 (MassLynx mass)
- $(M-H)^-$ *m/z* 321.0050 (UNIFI mass)

(Field Service Engineer to prepare)

1. Using a 5-mL pipette, add 5 mL of LC/MS grade methanol to the 50 mg vial of chloramphenicol.
2. Sonicate for five minutes and label as "10 mg/mL chloramphenicol" (expires in three month).

Sulfadimethoxine

- $C_{12}H_{14}N_4O_4S$
- (M+H) *m/z* 311.0814, (M-H) *m/z* 309.0658 (MassLynx masses)
- $(M+H)^+$ *m/z* 311.0809, $(M-H)^-$ *m/z* 309.0663 (UNIFI masses)

(Field Service Engineer to prepare)

1. Using a 1000-µL pipette, add 1000 µL of LC/MS grade methanol to the 5-mg vial of sulfadimethoxine.
2. Sonicate for five minutes and label as "5 mg/mL sulfadimethoxine" (expires in three month).

Sulfadimethoxine and chloramphenicol mix

1. Using a 200-µL pipette, add 100 µL of 5 mg/mL sulfadimethoxine to a 5-mL disposable glass vial.
2. Using a 200-µL pipette, add 100 µL of 10 mg/mL chloramphenicol to same vial.
3. Using a 1000-µL pipette, add 800 µL LC/MS grade methanol to the same vial and then recap and sonicate for five minutes.
4. Label as "500 ng (SDM) + 1 µg (Chlor)/µL stock solution" (expires in one month).
5. Using a 200-µL pipette, add 100 µL of the stock solution to a 5-mL disposable glass vial.
6. Using a 1000-µL pipette, add 900 µL of 10:90 acetonitrile/water to the same vial and then recap and sonicate for five minutes.
7. Label as "50 ng (SDM) + 100 ng (Chlor)/µL solution", (expires in one week).
8. Using a 200-µL pipette, add 100 µL of the 50 ng (SDM) + 100 ng (Chlor)/µL solution to a 5-mL disposable glass vial.
9. Using a 1000-µL pipette, add 900 µL of 10:90 acetonitrile/water to the same vial and then recap and sonicate for five minutes.
10. Label as "5 ng (SDM) + 10 ng (Chlor)/µL solution", (expires in one week).
11. Using a 200-µL pipette, add 100 µL of the 5 ng (SDM) + 10 ng (Chlor)/µL solution to a 5-mL disposable glass vial.
12. Using a 1000-µL pipette, add 900 µL of 10:90 acetonitrile/water to the same vial and then recap and sonicate for five minutes.
13. Label as "500 pg (SDM) + 1 ng (Chlor)/µL solution", (expires in one week).

Xevo G2-S/XS QTof/Tof

6 Mix tuning solution

1. Using a 1000-µL pipette, add 1000 µL of 6 Mix stock solution to a 10-mL volumetric flask.
2. Make up to 10 mL with LCMS diluent and sonicate for five minutes.
3. Label as "6 Mix Solution" and store in the refrigerator (expires in one month).

   NOTE: *This 6 Mix solution is used to make further dilutions for the LCMS system performance samples.*

4. Using a 1000-µL pipette, add 1000 µL of the "6 Mix solution" to a 10-mL volumetric flask.
5. Make up to 10 mL with the LCMS diluent, sonicate for five minutes.
6. Label as "6 Mix Tuning Solution 10 pg/µL SDM" and store in the refrigerator (expires in one week).

6 Mix solution - 5 pg/µL of sulfadimethoxine and 2.5 pg/µL chloramphenicol (Negative Ion sample)

1. Using a 200-µL pipette, add 100 µL of the "6 Mix solution" to a 2-mL ACQUITY vial.
2. Using a 1000-µL pipette add 1900 µL of LCMS diluent, recap and vortex the vial to mix fully.
3. Label the vial "6 Mix 5 pg/µL SDM" and store in the refrigerator (expires in one week).

6 Mix solution - 1 pg/µL of sulfadimethoxine and 0.5 pg/µL chloramphenicol (Positive Ion sample)

1. Using a 200-µL pipette, add 200 µL of the "6 Mix 5 pg/µL SDM" solution to a 2-mL ACQUITY vial.
2. Using a 1000-µL pipette add 800 µL of LCMS diluent, recap and vortex the vial to mix fully.
3. Label the vial "6 Mix 1 pg/µL SDM" and store in the refrigerator (expires in one week).

Sample preparation (700008893)

D(+)-Raffinose

- $C_{18}H_{32}O_{16}.5H_2O$
- (M+Na) *m/z* 527.1588, (M-H) *m/z* 503.1612 (MassLynx masses)
- $(M+Na)^+$ *m/z* 527.1583, $(M-H)^-$ *m/z* 503.1618 (UNIFI masses)

5 ng/µL raffinose solution

1. Using a 5-mL pipette, add 10 mL 50:50 acetonitrile/water to the contents of the 2.5 mg bottle of raffinose supplied in the sample kit and sonicate for five minutes.
2. Label as "250 ng/µL raffinose stock solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in three months).
3. Using a 1000-µL pipette, transfer 400 µL of the 250 ng/µL raffinose stock solution to a 20-mL volumetric flask.
4. Make up to 20 mL with 50:50 acetonitrile/water and sonicate for five minutes.
5. Label as "5 ng/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in two months).

500 pg/µL raffinose solution

1. Decant ~4 mL of the 5 ng/µL raffinose solution in 50:50 acetonitrile/water into a 5-mL glass vial.
2. Using a 1000-µL pipette, transfer 2000 µL of the 5 ng/µL raffinose solution to a 20-mL volumetric flask.
3. Make up to 20 mL with 50:50 acetonitrile/water and sonicate for five minutes.
4. Transfer from the 20-mL volumetric flask to a 30-mL fluidics container.
5. Label as "500 pg/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in one week).

Matrix (α-cyano-4-hydroxycinnamic acid)

- $C_{10}H_7N0_3$

1. Using a 5-mL pipette, add 2.7 mL of 50:50 TFA 0.1% aqueous/acetonitrile to the contents of the 10 ±0.1 mg vial of matrix and sonicate for five minutes.
2. Label as "3.6 mg/mL matrix solution" and store in the freezer (expires in one week).

Formic acid

(Vendor prepared, use as provided)

Aqueous ammonia

(Vendor prepared, use as provided)

Appendix

NOTE: *It is preferable to use micropipettes for volumes ≤1000 µL, and volumetric or graduated pipettes for volumes ≥1000 µL.*

Table 6: Air displacement micropipette volume ranges

Volume (µL)	Model	Operating ranges (µL)
1	Gilson or Eppendorf	0.5 to 1
20	Gilson or Eppendorf	5 to 20
200	Gilson or Eppendorf	50 to 200
1000	Gilson or Eppendorf	200 to 1000
5000	Gilson or Eppendorf	1000 to 5000

Table 7: Positive displacement micropipette volume ranges

Volume (µL)	Model	Operating ranges (µL)
10	Microman	1 to 10
25	Microman	3 to 25
50	Microman	20 to 50
100	Microman	10 to 100
250	Microman	50 to 250
1000	Microman	200 to 1000

Table 8: Volumetric pipette volume ranges

Volume (mL)	Model	Operating ranges (mL)
1	Volvac class A grade	0.01 to 1
2	Volvac class A grade	0.02 to 2
5	Volvac class A grade	0.05 to 5
10	Volvac class A grade	0.1 to 10
15	Volvac class A grade	0.15 to 15
20	Volvac class A grade	0.2 to 20
25	Volvac class A grade	0.25 to 25

Table 9: Graduated pipette volume ranges

Volume (mL)	Model	Operating ranges (mL)
1	Volvac class A grade	0.1 to 1
10	Volvac class A grade	1 to 10