Preparing Test Samples / TOF Instruments

This procedure describes how to prepare test stock solutions and diluted working solutions when required, from the original compounds supplied in the Waters Q-Tof product sample kits (p/n 700004768, and 700003276).

Fluidics systems may require larger volumes of sample to be created; scale the dilutions accordingly.

NOTE: *Bovine insulin, horse heart myoglobin, and haemoglobin are removed in the Waters Q-Tof Product Samples Kit (p/n 700004768) for countries where they are supplied locally due to import regulations.*

Parts required

Table 1: Q-Tof product sample kits

Part number	Description	Qty
700003276	Waters Q-Tof Product Sample Kit	1
700004768	Waters Q-Tof Product Sample Kit (without Bovine Insulin and Horse Heart Myoglobin)	1

NOTE: *The serial numbers for sample kit components differ between sample kits p/n 700003276 and 700004768.*

Table 2: Sample kit 700003276 components

Serial	Sample	Supply format	Amount required in each vial	Number of vials
1	Leucine Enkephalin	Powder	3 ±0.15 mg	2
2	Sodium Iodide	Solution	25 mL	1
3	Bovine Insulin	Powder	3 ±0.15 mg	1
4	Horse Heart Myoglobin	Powder	2.5 ±0.125 mg	2
5	D(+)-Raffinose	Powder	2.5 ±0.125 mg	2
6	[Glu^1]-Fibrinopeptide B	Powder	0.1 ±0.01 mg	2
7	Sodium Hydroxide	Solution	2 mL	1
8	Haemoglobin (Human)	Powder	2.52 ±0.125 mg	1
9	Matrix	Powder	10 ±0.1mg	5
10	PEG Mix	Ready mixed (soln)	2 mL	1
11	PEG MALDI	Ready mixed (soln)	2 mL	1

Table 3: Sample kit 700004768 components

Serial	Sample	Supply format	Amount required in each vial	Number of vials
1	Leucine Enkephalin	Powder	3 ±0.15 mg	2
2	Sodium Iodide	Solution	25 mL	1
4	D(+)-Raffinose	Powder	2.5 ±0.125 mg	2
5	[Glu1]-Fibrinopeptide B	Powder	0.1 ±0.01 mg	2
6	Sodium Hydroxide	Solution	2 mL	1
8	Matrix	Powder	10 ±0.1 mg	5
9	PEG Mix	Ready mixed (soln)	2 mL	1
10	PEG MALDI	Ready mixed (soln)	2 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 or deionized water

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

Additives

- >99.5% purity Glacial acetic acid
- >98% purity formic acid
- Ammonia solution (specific gravity 0.88)
- ≥99% HPLC-grade trifluoroacetic acid

CAUTION: ***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)
- 5-mL disposable glass vials (with caps)
- 30-mL Nalgene fluidics container (with caps)
- 100-mL graduated measuring cylinder

CAUTION: ***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

CAUTION: ***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.***

CAUTION: ***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 methanol/water + 1% acetic acid

1. Using a 100-mL measuring cylinder, transfer 100 mL LCMS grade methanol to a 250-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 100 mL deionized or LCMS grade water to the 250-mL Duran bottle.
3. Using a 1000-µL pipette, add 2000 µL Glacial acetic acid to the 250-mL Duran bottle.
4. Sonicate for 10 minutes and label as "50:50 methanol/water + 1% acetic acid".

50:50 methanol/water + 1% ammonia

1. Using a 100-mL measuring cylinder, transfer 25 mL LCMS-grade methanol to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 25 mL deionized or LCMS grade water to a 100-mL Duran bottle.
3. Using a 1000-µL pipette, add 500 µL ammonia solution to the 100-mL Duran bottle.
4. Sonicate for 10 minutes and label as "50:50 methanol/water + 1% ammonia".

50:50 acetonitrile/water

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 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 water to the 100-mL Duran bottle.
3. Using a 100-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 acetonitrile/water + 0.2% 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 water to the 100-mL Duran bottle.
3. Using a 1000-µL pipette, add 200 µL formic acid to the 100-mL Duran bottle.
4. Sonicate for 10 minutes and label as "50:50 acetonitrile/water + 0.2% formic acid".

90:10 2-propanol/water

1. Using a 100-mL measuring cylinder, transfer 45 mL HPLC grade 2-propanol to a 100-mL Duran bottle.
2. Using a 100-mL measuring cylinder, transfer 5 mL deionized or LCMS grade water to the 100-mL Duran bottle.
3. Sonicate for 10 minutes and label as "90:10 2-propanol/water".

50:50 2-propanol/water

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

Trifluoroacetic acid 0.1% aqueous

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 Trifluoroacetic acid to the 100-mL Duran bottle.
3. Sonicate for 10 minutes and label as "TFA 0.1% aqueous".

50:50 Trifluoroacetic acid 0.1% aqueous/acetonitrile

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".

Matrix (α-cyano-4-hydroxycinnamic acid)

- $C_{10}H_7NO_3$

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 5 minutes.
2. Label as "3.6 mg/mL matrix solution" and store in the freezer (expires in 1 week).

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

2 ng/µL leucine enkephalin

1. Using a 5-mL pipette, add 7.5 mL of water to the contents of a 3 ±0.15 mg bottle of leucine enkephalin.
2. Recap, shake well, and sonicate for 5 minutes.
3. Label as "400 ng/µL leucine enkephalin in water" and store in the freezer (expires in 3 months).
4. Using a 200-µL pipette, transfer 100 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 5 minutes.
6. Label as "2 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the freezer (expires in 1 month).
7. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "2 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the freezer (expires in 1 month).

200 pg/µL leucine enkephalin

1. Decant ~4 mL of the 2 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid solution into a 5-mL glass vial.
2. Using a 1000-µL pipette, transfer 2000 µL of the 2 ng/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid solution to a 20-mL volumetric flask.
3. Make up to 20 mL with 50:50 acetonitrile/water + 0.1% formic acid and sonicate for 5 minutes.
4. Label as "200 pg/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the refrigerator (expires in 2 weeks).
5. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "200 pg/µL leucine enkephalin in 50:50 acetonitrile/water + 0.1% formic acid" and store in the refrigerator (expires in 2 weeks).

50 pg/µL leucine enkephalin

1. Using a 1000 µL pipette, transfer 500 µL of the 2 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 5 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 1 week).

Sodium iodide

(Vendor prepared, use as provided)

1. Dissolve 50 ±0.2 mg of sodium iodide in 25 mL of 50:50 2-propanol/water and sonicate for 5 minutes.
2. Label as "2 µg/µL sodium iodide solution", this can be stored on the bench (expires 3 months after opening).

Bovine insulin

- $C_{254}H_{377}N_{65}O_{75}S_6$
- Mass 5733.49 Da

NOTE: *Bovine insulin is removed in the Waters Q-Tof Product Samples Kit (p/n 700004768) for countries where bovine insulin is supplied locally due to import regulations.*

50 pmol/µL bovine insulin

1. Using a 1000-µL pipette, add 5250 µL of methanol and add 5250 µL of water to the contents of a 3 ±0.15 mg bottle of bovine insulin and sonicate for 5 minutes.
2. Label as "50 pmol/µL bovine insulin solution" and store in the refrigerator (expires in 3 months).

NOTE: *The bovine insulin will not dissolve completely until the acid or ammonia is added.*

5 pmol/µL bovine insulin solution + 1% acetic acid

1. Using a 1000-µL pipette, transfer 2000 µL of the 50 pmol/µL bovine insulin solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 methanol/water + 1% acetic acid and sonicate for 5 minutes.
3. Label as "5 pmol/µL bovine insulin solution in 1% acetic acid" and store in the refrigerator (expires in 1 month).
4. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "5 pmol/µL bovine insulin solution in 1% acetic acid" and store in the refrigerator (expires in 1 month).

500 fmol/µL bovine insulin solution + 1% acetic acid

1. Using a 1000-µL pipette, transfer 300 µL of the "5 pmol/µL bovine insulin solution + 1% acetic acid" to a 5-mL glass vial.
2. Using a 5-mL pipette, add 2.7 mL of 50:50 methanol/water + 1% acetic acid and sonicate for 5 minutes.
3. Label as "500 fmol/µL bovine insulin solution in 1% acetic acid" and store in the refrigerator (expires in 1 week).

5 pmol/µL bovine insulin solution + 1% ammonia

1. Using a 1000-µL pipette, transfer 2000 µL of the 50 pmol/µL bovine insulin solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 methanol/water + 1% ammonia and sonicate for 5 minutes.
3. Label as "5 pmol/µL bovine insulin solution in 1% ammonia" and store in the refrigerator (expires in 1 week).
4. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "5 pmol/µL bovine insulin solution in 1% ammonia" and store in the refrigerator (expires in 1 week).

Horse heart myoglobin

- Mass 16952 Da

NOTE: *Horse heart myoglobin is removed from the Waters Q-Tof product samples kits (p/n 700004734 and 715004768) for countries where horse heart myoglobin is supplied locally due to import regulations.*

200 fmol/µL horse heart myoglobin solution

1. Using a 5-mL pipette, add 15 mL of 50:50 acetonitrile/water + 0.2% formic acid to the contents of a 2.5 ±0.125 mg bottle of horse heart myoglobin and sonicate for 5 minutes.
2. Label as "10 pmol/µL horse heart myoglobin solution" and store in the freezer (expires in 1 month).
3. Using a 200-µL pipette, transfer 50 µL of 10 pmol/µL horse heart myoglobin to a 5-mL glass vial.
4. Using a 1000-µL pipette, add 2000 µL 50:50 acetonitrile/water + 0.2% formic acid to the 5-mL glass vial and using a 200-µL pipette, add 450 µL of 50:50 acetonitrile/water + 0.2% formic acid.
5. Sonicate for 5 minutes and label as "200 fmol/µL horse heart myoglobin solution". Store in the freezer (expires in 1 week).

D(+)-Raffinose

- $C_{18}H_{32}O_{16}.5H_2O$
- $(M-H)^-$ M/Z 503.1612, $(M+Na)^+$ M/Z 527.1588

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 ±0.125 mg bottle of raffinose supplied in the sample kit and sonicate for 5 minutes.
2. Label as "250 ng/µL raffinose stock solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 3 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 5 minutes.
5. Label as "5 ng/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 2 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 5 minutes.
4. Label as "500 pg/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 1 week).
5. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "500 pg/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 1 week).

250 pg/µL raffinose solution

1. Decant ~2 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 1000 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 5 minutes.
4. Label as "250 pg/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 1 week).
5. For Fluidics systems, transfer from the 20-mL volumetric to a 30-mL fluidics container and label as "250 pg/µL raffinose solution in 50:50 acetonitrile/water" and store in the refrigerator (expires in 1 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, $(M+2H)^{2+}$ M/Z 785.8426, $(M-H)^{-}$ M/Z 1568.6618

NOTE: *Many proteins are "sticky" by nature, to avoid material adhering to the outside of the pipette, do not immerse the pipette tip any further than necessary to retrieve the 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 ±0.01 mg bottle of GluFib and sonicate for 5 minutes.
2. Label as "32 pmol/µL GluFib stock solution" and store in the freezer (expires in 3 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 methanol/water + 1% acetic acid and using a 200-µL pipette, add 100 µL of 50:50 methanol/water + 1% acetic acid to the 5-mL glass vial.
5. Sonicate for 5 minutes and label as "1 pmol/µL GluFib solution 50:50 methanol/water + 1% acetic acid". Store in the freezer (expires in 1 week).

500 fmol/µL GluFib solution

1. Using a 1000-µL pipette, transfer 2000 µL of the 1 pmol/µL GluFib solution to a 5-mL glass vial.
2. Using a 1000-µL pipette, add 2000 µL of 50:50 methanol/water + 1% acetic acid to the 5-mL glass vial.
3. Label as "500 fmol/µL GluFib solution in 50:50 methanol/water + 1% acetic acid" and store in the freezer (expires in 1 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 methanol/water + 1% acetic acid and sonicate for 5 minutes.
3. Label as "100 fmol/µL GluFib solution in 50:50 methanol/water + 1% acetic acid" and store in the freezer (expires in 1 week).
4. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "100 fmol/µL GluFib solution in 50:50 methanol/water + 1% acetic acid" and store in the freezer (expires in 1 week).

50 fmol/µL GluFib solution

1. Using a 1000-µL pipette, transfer 1000 µL of the 1 pmol/µL GluFib solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 methanol/water + 1% acetic acid and sonicate for 5 minutes.
3. Label as "50 fmol/µL GluFib solution in 50:50 methanol/water + 1% acetic acid" and store in the freezer (expires in 1 week).
4. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "50 fmol/µL GluFib solution in 50:50 methanol/water + 1% acetic acid" and store in the freezer (expires in 1 week).

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 5 minutes and label as "1 pmol/µL GluFib solution in 0.1% TFA". Store in the freezer (expires in 1 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 5 minutes.
6. Label as "200 fmol/µL GluFib solution in 0.1% TFA" and store in the freezer (expires in 1 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 5 minutes.
3. Label as "20 fmol/µL GluFib solution in 0.1% TFA" and store in the freezer (expires in 1 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.

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

Sodium hydroxide to make "sodium formate"

(Vendor prepared, use as provided)

- 0.1 M sodium hydroxide in water

(Field Service Engineer to prepare)

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

1. Using a 1000-µL pipette, transfer 1000 µL of 0.1 M sodium hydroxide solution to a 20-mL volumetric flask.
2. Using a 1000-µL pipette, transfer 900 µL of deionized or LCMS-grade water to the 20-mL volumetric flask.
3. Using a 200-µL pipette, add 100 µL formic acid to the 20-mL volumetric flask.
4. Make up to 20 mL with 90:10 2-propanol/water and sonicate for 5 minutes.
5. Label as "5 mM sodium formate solution in 90:10 2-propanol/water" and store in the refrigerator (expires in 1 week).
6. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "5 mM sodium formate solution in 90:10 2-propanol/water" and store in the refrigerator (expires in 1 week).

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

1. Using a 1000-µL pipette, add 2000 µL of 5 mM sodium formate solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 90:10 2-propanol/water and sonicate for 5 minutes.
3. Label as "0.5 mM sodium formate solution in 90:10 2-propanol/water" and store in the refrigerator (expires in 1 week).
4. For Fluidics systems, transfer from the 20-mL volumetric flask to a 30-mL fluidics container and label as "0.5 mM sodium formate solution in 90:10 2-propanol/water" and store in the refrigerator (expires in 1 week).

Haemoglobin (human)

- α-globin 15126.38 Da, β-globin 15867.2 Da

10 pmol/µL haemoglobin solution

1. Using a 5-mL pipette, add 14.4 mL of 50:50 acetonitrile/water + 0.2% formic acid to the contents of a 2.52 ±0.125 mg bottle of haemoglobin (human) and sonicate for 5 minutes.
2. Label as "10 pmol/µL haemoglobin solution" and store in the refrigerator (expires in 1 week).

PEG Mix

(Vendor prepared, use as provided)

PEG Mix stock solution

NOTE: *Individual PEG 200, 400, and 600 are not supplied in the kit (for reference only).*

1. Mix 40 mg PEG 200, 50 mg PEG 400, 80 mg PEG 600, and 150 mg ammonium acetate in 100 mL of 50:50 acetonitrile/water + 0.2% formic acid.
2. Sonicate for 5 minutes and label as "PEG Mix stock solution". This can be stored on the bench (expires 3 months after opening).

Specification solution

1. Using a 200-µL pipette, transfer 200 µL of PEG Mix stock solution to a 20-mL volumetric flask.
2. Make up to 20 mL with 50:50 acetonitrile/water + 0.2% formic acid.
3. Sonicate for 5 minutes and label as "PEG Mix specification solution". This can be stored on the bench (expires in 3 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 5 minutes and label as "PEG MALDI solution”. This can be stored on the bench (expires 3 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" preparation) and sonicate for 5 minutes.
3. Label as "PEG MALDI specification solution (with sodium iodide)" and store in the refrigerator (expires in 1 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.

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

Appendix

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

Table 4: 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 5: 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 6: 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 7: 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