Sample Preparation Instructions

Electrospray:	Electrospray Ionization (ESI) is a mild solution based ionization process which relies on the analyte having either a basic or acidic site for ionization.
Vial type:	2ml glass vials.
Lid:	Snap-cap with Teflon disk.
Solvent:	HPLC grade acetonitrile, methanol, water, chloroform, dichloromethane or mixtures of these.
Volume:	>1000 µl, if less, then use vial inserts.
Concentration:	<2000 Da use 1µg ml^{-1} >2000 Da use 100µg ml^{-1}.
Caution:	You must not use THF or DMF as solvent. ESI is severely compromised by the presence of involatile buffers and involatile salts. These must be removed from the sample or substituted by a volatile option, e.g. ammonium acetate. All sample need to be particulate free so filter when necessary.

Contamination Peaks in Positive Ion ESI.

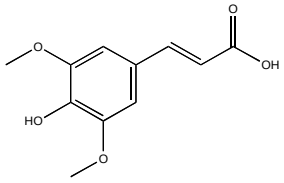
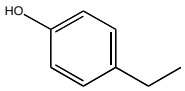
m/z	Ion	Compound
42	$(M+H)^+$	Acetonitrile
59	$(M+NH_4)^+$	Acetonitrile
64	$(M+Na)^+$	Acetonitrile
79	$(M+H)^+$	DMSO
83	$(2M+H)^+$	Acetonitrile
101	$(M+Na)^+$	DMSO
102	$(M+H)^+$	Triethylamine (TEA)
104/106	$(M+Cu)^+$	Acetonitrile
105	(2M+Na)+	Acetonitrile
120	$(M+Na+CH_3CN)^+$	DMSO
122	$(M+H)^+$	Tris
123	$(M+H)^+$	Dimethylaminopyridine (DMAP)
130	$(M+H)^+$	Diisopropylethylamine (DIPEA)
144	$(M+H)^+$	Tripropylamine
145/147	$(2M+Cu)^+$	Acetonitrile
146	$(3M+Na)^+$ Diisooctyl.Phthalate	Acetonitrile
150	$(M+H)^+$	Phenyldiethylamine
157	$(2M+H)^+$	DMSO
159	$(M+H)^+$	Sodiu7.2t2336 5271576 0

Elute the Peptides or Proteins:

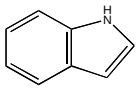
For Zip-Tip C18 and Zip-Tip C4 pipette tips, dispense 1 to 4 µl of *elution solution* into a clean vial.

Sample Loading: Typically take 2µl of sample in the pmol/µl concentration range and mix with 2µl of matrix solution in the bottom of a 0.5ml eppendorf tube. Take 2µl of this mixture and apply to the sample position. Repeat this for the desired number of samples and allow the target to dry.

Matrix Solutions:

Name	Structure	Substrate
3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic Acid)		Proteins, peptides, polymers
Alpha-cycano-4-hydroxycinnamic acid (Alpha cyano)		

Beta Indole acrylic acid (IAA)



Preparation of tryptic digests for MALDI-TOF analysis using Coomassie Blue stained gel slices.

Dehydrate Gel Slices.

1. Equilibrate the stained gel slice in 50mM ammonium bicarbonate.
2. Cut the slice into 1 x 1 mm pieces.
3. Place pieces in 50% acetonitrile: 50% 50mM ammonium bicarbonate, (from a 100mM stock of ammonium bicarbonate).
4. Replace supernatant with acetonitrile to completely dehydrate.
5. Remove acetonitrile in a Speed Vac.

Tryptic digest.

6. Rehydrate slices in 25mM ammonium bicarbonate which contains 50-100ng trypsin per 20ul volume. Include ~0.01% octylglucoside. Add sufficient volume to swell slices to original volume and then excess 25mM ammonium bicarbonate to cover completely. (Use sequencing grade trypsin – Promega or Roche)
7. Incubate overnight at 37 ^{o}C. Remove supernatant and save for analysis.
8. Rinse slices with 80% acetonitrile, 1% formic acid. Slices will shrink.
9. Remove supernatant and pool with supernatant from step 8.
10. Dry down digest in Speed Vac. Stop when digest is almost dry.
11. Resuspend digest in a small volume of 1% formic if necessary.
12. Desalt on a Millipore Zip-Tip (see above) according to instructions.

Sample is ready for MALDI-TOF analysis.

Trypsin Autolysis Peaks which can be used for internal calibration:

m/z
842.508
1045.564
2211.108
2225.119