

# 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  $\mu$ l, if less, then use vial inserts.

Concentration: <2000 Da use 1 $\mu$ g ml<sup>-1</sup>

>2000 Da use 100 $\mu$ g ml<sup>-1</sup>.

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) <sup>+</sup>                     | Acetonitrile                       |
| 59      | (M+NH <sub>4</sub> ) <sup>+</sup>      | Acetonitrile                       |
| 64      | (M+Na) <sup>+</sup>                    | Acetonitrile                       |
| 79      | (M+H) <sup>+</sup>                     | DMSO                               |
| 83      | (2M+H) <sup>+</sup>                    | Acetonitrile                       |
| 101     | (M+Na) <sup>+</sup>                    | DMSO                               |
| 102     | (M+H) <sup>+</sup>                     | Triethylamine (TEA)                |
| 104/106 | (M+Cu) <sup>+</sup>                    | Acetonitrile                       |
| 105     | (2M+Na) <sup>+</sup>                   | Acetonitrile                       |
| 120     | (M+Na+CH <sub>3</sub> CN) <sup>+</sup> | DMSO                               |
| 122     | (M+H) <sup>+</sup>                     | Tris                               |
| 123     | (M+H) <sup>+</sup>                     | Dimethylaminopyridine (DMAP)       |
| 130     | (M+H) <sup>+</sup>                     | Diisopropylethylamine (DIPEA)      |
| 144     | (M+H) <sup>+</sup>                     | Tripropylamine                     |
| 145/147 | (2M+Cu) <sup>+</sup>                   | Acetonitrile                       |
| 146     | (3M+Na) <sup>+</sup>                   | Acetonitrile                       |
| 150     | (M+H) <sup>+</sup>                     | Phenyldiethylamine                 |
| 157     | (2M+H) <sup>+</sup>                    | DMSO                               |
| 159     | (M+H) <sup>+</sup>                     | Sodium Trifluoroacetate            |
| 169     | (2M+H) <sup>+</sup>                    | d6-DMSO                            |
| 179     | (2M+Na) <sup>+</sup>                   | DMSO                               |
| 186     | (M+H) <sup>+</sup>                     | Tributylamine                      |
| 214     | (M+H) <sup>+</sup>                     | Unidentified contaminant           |
| 225     | (M+H) <sup>+</sup>                     | Dicyclohexyl Urea (DCU)            |
| 242     | M <sup>+</sup>                         | Tetrabutylammonium                 |
| 243     | M <sup>+</sup>                         | Trityl Cation                      |
| 257     | (3M+H) <sup>+</sup>                    | DMSO                               |
| 273     | M <sup>+</sup>                         | Monomethoxytrityl Cation (MMT)     |
| 279     | (M+H) <sup>+</sup>                     | Dibutylphthalate (Plasticiser)     |
| 301     | (M+Na) <sup>+</sup>                    | Dibutylphthalate (Plasticiser)     |
| 317     | (M+K) <sup>+</sup>                     | Dibutylphthalate (Plasticiser)     |
| 338     | (M+H) <sup>+</sup>                     | Erucamide                          |
| 360     | (M+Na) <sup>+</sup>                    | Erucamide                          |
| 391     | (M+H) <sup>+</sup>                     | Diisooctyl Phthalate (Plasticiser) |
| 413     | (M+Na) <sup>+</sup>                    | Diisooctyl Phthalate (Plasticiser) |
| 429     | (M+K) <sup>+</sup>                     | Diisooctyl Phthalate (Plasticiser) |
| 449     | (2M+H) <sup>+</sup>                    | Dicyclohexyl Urea (DCU)            |
| 454     | (M+Na+CH <sub>3</sub> CN) <sup>+</sup> | Diisooctyl Phthalate (Plasticiser) |
| 798     | (2M+NH <sub>4</sub> ) <sup>+</sup>     | Diisooctyl Phthalate (Plasticiser) |
| 803     | (2M+Na) <sup>+</sup>                   | Diisooctyl Phthalate (Plasticiser) |

**Ions observed: Positive ion mode for molecules that possess a basic site.**

|              |                             |
|--------------|-----------------------------|
| $(M+H)^+$    | $(M+1)$                     |
| $(M+NH_4)^+$ | $(M+18)$                    |
| $(M+Na)^+$   | $(M+23)$                    |
| $(M+K)^+$    | $(M+39)$                    |
| $M^+$        | Quaternary ammonium species |

Ions separated by 5amu suggests you have  $(M+NH_4)^+$  and  $(M+Na)^+$ . Look for accompanying  $(M+H)^+$  and  $(M+K)^+$ .

Similarly if you suspect  $(M+Na)^+$  then look for  $(M+K)^+$  at 16 Da higher than  $(M+Na)^+$

You may also check for multimers if you see higher mass peaks e.g.  $(2M+H)^+$ ,  $(2M+NH_4)^+$ ,  $(2M+Na)^+$ ,  $(3M+H)^+$  etc.

$(M+CH_3CN)^+$  and  $(M+CH_3OH)^+$  are also sometimes observed (+41 & +32 Da)

Generally multimers are observed if the sample is too concentrated, though some molecules “self assemble” to give higher mass species.

**Ions observed: Negative ion mode for molecules that possess an acidic site.**

|           |           |
|-----------|-----------|
| $(M-H)^-$ | $(M-1)^-$ |
|-----------|-----------|

Deprotonation can be aided by addition of a trace amount of base,  $NH_4OH$ , TEA, TPA

Formic acid and TFA adducts are also often observed:

|                 |             |
|-----------------|-------------|
| $(M+HCOOH-H)^-$ | $(M+45)^-$  |
| $(M+TFA-H)^-$   | $(M+113)^-$ |

|                |  |
|----------------|--|
| <b>MALDI:</b>  | The sample is mixed with a UV absorbing matrix, the latter in great excess. Singly charged species are product.  |
| Vial Type:     | Eppendorf  |
| Solvent:       | HPLC grade acetonitrile, methanol, water or mixtures of these for biomolecules and DCM or THF for polymers.      |
| Concentration: | <5000Da use 1-10 pmol $\mu\text{l}^{-1}$<br>>5000Da use 10-100 pmol $\mu\text{l}^{-1}$                           |
| Caution:       | Sensitivity and resolution are compromised by the presence of salts. Rigorous sample clean up improves analysis. |

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#### **Zip-Tip reversed phase sample preparation:**

|                         |   |
|-------------------------|---|
| Sample preparation:     | Adjust sample to 0.1% TFA, final pH should be <4. |
| Equilibration Solution: | 0.1% TFA in Milli-Q grade water.                  |
| Wash Solution:          | 0.1% TFA in Milli-Q grade water.                  |
| Elution Solution:       | 0.1% TFA / 50% AcN.                               |

#### ***Equilibrate the Zip-Tip pipette for sample binding:***

1. Depress pipettor plunger to a dead stop. Using the maximum volume setting of 10 $\mu\text{l}$ , aspirate *wetting solution* into the tip. Dispense to waste. Repeat.
2. Aspirate equilibration solution. Dispense to waste. Repeat.

#### ***Bind and Wash the Peptides or Proteins:***

Follow these steps after equilibrating the Zip-Tip pipette tip.

1. Bind peptides and/or proteins to Zip-Tip pipette tip by fully depressing the pipette plunger to a dead stop. Aspirate and dispense the sample 7-10 cycles for maximum binding of complex mixtures.
2. Aspirate *wash solution* into tip and dispense to waste. Repeat at least once.

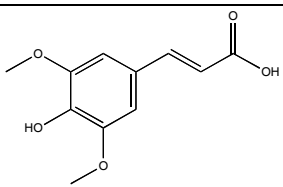
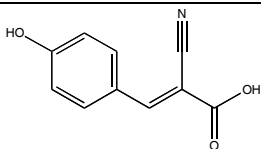
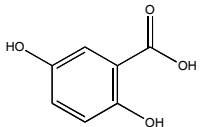
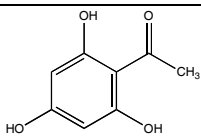
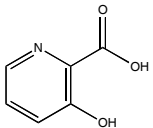
NOTE: A 5% methanol in 0.1% TFA / Water wash can improve desalting efficiency. Additional washing may be required for electrospray MS.

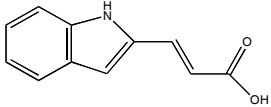
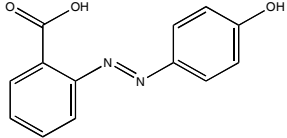
### ***Elute the Peptides or Proteins:***

For Zip-Tip C18 and Zip-Tip C4 pipette tips, dispense 1 to 4  $\mu\text{l}$  of *elution solution* into a clean vial.

**Sample Loading:** Typically take 2  $\mu\text{l}$  of sample in the pmol/ $\mu\text{l}$  concentration range and mix with 2  $\mu\text{l}$  of matrix solution in the bottom of a 0.5ml eppendorf tube. Take 2  $\mu\text{l}$  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:**

| <b>Name</b>  | <b>Structure</b>  | <b>Substrate</b>                          |
|--|---|---|
| 3,5-dimethoxy-4-hydroxycinnamic acid<br>(Sinapinic Acid) |   | Proteins, peptides, polymers              |
| Alpha-cyano-4-hydroxycinnamic acid<br>(Alpha cyano)      |  | Peptides, polymers                        |
| 2,5-Dihydroxybenzoic acid<br>(DHB)                       |  | Sugars, peptides, nucleotides, polymers   |
| 2,4,6-Trihydroxyacetophenone<br>(THAP)                   |  | Oligonucleotides                          |
| Hydroxypicolinic acid<br>(HPA)                           |  | Oligonucleotides, peptides, glycoproteins |

|   |   |                                 |
|---|---|---------------------------------|
| Beta Indole acrylic acid (IAA)            |  | Polymethyl methacrylates        |
| 2-(4-Hydroxyphenylazo)benzoic acid (HABA) |  | Glycolipids, peptides, proteins |

### Preparation of Standards for MALDI:

Prepare standards at 1mg/ml in 0.1% TFA in Water and do appropriate dilutions to obtain a final concentration of approximately 10 pmol/ $\mu$ l

| Substance Name    | Average Molecular Mass (amu) | Dilution factor of 1mg/ml solution to give 10 pmol/ $\mu$ l |
|-------------------|------------------------------|---|
| Angiotensin I     | 1296.5                       | 100   |
| ACTH (18-39 clip) | 2465.7                       | 40  |
| Insulin (Bovine)  | 5733.5                       | 20  |

### Suggested tolerated contaminant concentrations:

| Contaminant                 | Concentration (Maximum) |
|-----------------------------|-------------------------|
| Phosphate buffer            | 20mM                    |
| Tris buffer                 | 50mM                    |
| Detergents                  | 0.1%                    |
| SDS                         | 0.01%                   |
| Alkali metal salts          | 1M                      |
| Glycerol                    | 2%                      |
| NH <sub>4</sub> bicarbonate | 30mM                    |
| Guanidine                   | 1M                      |
| Sodium azide                | 1%                      |

**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 °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:***

| <b>m/z</b> |
|------------|
| 842.508    |
| 1045.564   |
| 2211.108   |
| 2225.119   |