## **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 1µg ml<sup>-</sup>1

>2000 Da use 100µg ml<sup>-</sup>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                  | Acetonitrile |  |
| 79      | $(M+H)^+$                              | DMSO                          |              |  |
| 83      | $(2M+H)^{+}$                           | Acetonitrile                  |              |  |
| 101     | $(M+Na)^+$                             | DMSO                          |              |  |
| 102     | (M+II) <sup>+</sup>                    | Triethylamine (TEA)           |              |  |
| 104/106 | $(M+Cu)^+$                             | Acetonitrile                  |              |  |
| 105     | (2M+Na)+                               | Acetonitrile                  |              |  |
| 120     | (M+Na+CH <sub>3</sub> CN) <sup>+</sup> | DMSO                          |              |  |
| 122     | $(M+H)^+$                              | Tris                          |              |  |
| 123     | $(M+H)^+$                              | Dimethylaminopyridine (DMAP)  |              |  |
| 130     | $(M+H)^+$                              | Diisopropylethylamine (DIPEA) |              |  |
| 144     | $(M+H)^+$                              | Tripropylamine                |              |  |
| 145/147 | (2M+Cu) <sup>+</sup>                   | Acetonitrile                  |              |  |
| 146     | (3M+Na)isooctyl.Phthalate              | Acetonitrile                  |              |  |
| 150     | $(M+H)^+$                              | Phenyldiethylamine            |              |  |
| 157     | (2M+H) <sup>+</sup>                    | DMSO                          |              |  |
| 159     | $\left( M+H\right) ^{+}$               | Sodiu7.2t2336 5271576         |              |  |

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### Elute the Peptides or Proteins:

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

**Sample Loading:** 

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

| Name  | Structure | Substrate                    |
|---|-----------|------------------------------|
| 3,5-dimethoxy-4-hydroxycinnamic acid (Sinapinic Acid) | ОН        | Proteins, peptides, polymers |

Alpha-cycano-4hydroxycinnamic 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 °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 |