Sample Preparation for MALDI-TOF

Samples for MALDI-TOF analysis need to meet certain requirements for obtaining good spectra. The more careful you prepare samples (including early steps of isolation and preparation) the more likely a successful analysis will be. Here are some guidelines of which kind of treatment is advantageous for mass spectrometric analysis and which is not:

- ► Avoid the use of non-volatile agents like salts (NaCl, CaCl2, KH2PO4), detergents (Tween, Triton, SDS), chaotropic agents (Urea, Guanidinium salts) and solvents like DMSO or Glycerol.

- ► If you can't avoid these agents, purify. Dialysis and RP-HPLC are good purification methods if you use volatile solvents and buffers. After purification, lyophilize if possible. Ion exchange beads may work well for salt removal.

- ► Suitable solvents are ones that are volatile. For sample work up and purification: water, ammonium hydrocarbonate, ammonium acetate, ammonium formiate, acetonitrile, trifluoroacetic acid.

- ► Quantitate the sample you are going to provide for analysis by methods like: photometry (OD), and ELISA. HPLC is useful since it allows for purification and quantitation in a single procedure. The range for many samples/preparations is not very large,

therefore it is necessary to have a good estimate of the sample amount because the sample amount may need to be varied on the target.

► The total amount of sample needed for MALDI analysis depends on the sample type.

► Give information like: structure, sequence, molecular weight,type of compound, biological activity, chemical reactivity, pH, sample amount/concentration, describe purification/isolation with focus on relative agents/solvents, known or suspected impurities, suitable solvents, hazardous properties: radioactivity, carcinogenicity, poison, or explosive.

For removing contaminants and interfering substances you can used : Microcon centrifugal filteration (3 kDa cut-off), Mini-dialysis, TCA acetone precipitation, C18 or C4 micro-reversed-phase chromatography (Zip tipping), and Amersham 2D clean-up. When submitting samples, please use a sample container of appropriate size. Two µL of sample is more easily recovered from 0.5mL Eppendorf tubes than from 1.5mL . Prerinsing the tube with methanol or acetonitrile lowers the chemical background for low concentration samples or complex mixtures.

Table shows the *MAXIMUM* concentration of surfactants, buffers and salts permissible in MALDI. ***N/A*** means that technique is not compatible with *ANY* amount of indicated compound. (Modified from "The Expanding Role of Mass Spectrometry in Biotechnology" by Gary Siuzdak, 2003, p. 84-85)

Surfactants, Buffers, Salts	Concentation, mM
TRIS	100
HEPES	*100*
BICINE	*50*
Urea	*500*
Guanidine, HCl	*250*
Dithiothreitol	*500*
Glycerol	*130*
n-Octyl-b-glucopyranoside	*3.4*
n-Octyl-sucrose	*N/A*
n-Dodecyl sucrose	*N/A*
n-Dodecyl maltoside	*N/A*
Octyl thioglucoside	*N/A*
n-Hexyl glucoside	*N/A*
n-Dodecyl glucoside	*N/A*
PEG1000	*N/A*
PEG2000	*0.5*
Triton X-100	*1.6*
NP-40	*1.7*
Zwittergent, 3-16	*2.6*
Tween20	*N/A*
Thesit	*N/A*
SDS	*0.35*
LDAO	*4.4*
CTAB	*N/A*
CHAPS	*0.16*
Sodium Cholate	*N/A*
Sodium Taurocholate	*N/A*
Sodium Azide	*15*
NH4CHO3	*50*
NaCl	*50*
Sodium Acetate	*50*
NaHPO4	*10*
TFA	*N/A*