Instructions for making peptide/small protein MALDI spots with a-cyano-4-hydroxycinnamic acid (CCA or CHCA)

Weight out about one milligram or three of recrystallized CCA into a small eppendorf tube

Compute final volume, final CCA concentration should be 10 g/L

Add half of the computed volume of acetonitrile to the CCA powder

Add 49% of the computed volume of HPLC-grade water

Add 1% of the computed volume of 10% v/v trifluoroacetic acid (TFA).

Mix well, make sure all of the matrix powder dissolves

Combine 5 parts CCA solution with 1 part of acidified (very important) sample solution. If the solution turns yellow, add a small amount of 1% v/v TFA. Solution must be clear prior to depositing on MALDI target

Mix well and deposit 1 µl of this solution onto the target

Allow spot to dry in air, a proper CCA spot should be flat, yellow, and opaque in appearance. If the spot is transparent add some TFA to the matrix/analyte mixture and try spotting again.

Use a laser attenuation of 45-50 for most samples.

alpha-cyano-4-hydroxycinnamic acid
a.k.a: CCA or CHCA
Molecular Weight =189.1722
Exact Mass =189.04259
Molecular Formula =C_{10}H_{7}NO_{3}