

EAM Selection and Preparation – Peptide and Protein Detection

Ciphergen has three different EAM compounds for the detection of proteins and peptides. The general guidelines for choosing an EAM are based on the MW and chemical nature of the analyte, but there are no absolute rules.

CHCA Alpha-cyano-4-hydroxy cinnamic acid, MW=189.2. **CHCA** is especially good for small molecules, <15 kD. Use a saturated solution for molecules between 2-15 kDa, and a 1/2-1/5 dilution of the saturated solution for anything smaller (dilution can be adjusted according to need). For small compounds, the saturated **CHCA** can be diluted up to 1/50 in solvent, and 0.5µl/spot used.

SPA Sinapinic acid, MW=224.2. This compound is recommended for all larger proteins, but also works reasonably well for peptides. Use a saturated solution of SPA. Two additions of 0.5µl/spot are recommended. Let the SPA air dry between additions. In general, SPA gives better resolution and less multiply charged ions than EAM-1.

EAM-1 This compound works very well for proteins >15 kDa. Use a saturated solution or a 2-fold dilution (in solvent). Since peaks from EAM-1 tend to be broader (due to more adduct formation), use calibrants (external or internal) detected with EAM-1, not SPA or CHCA, for best mass accuracy.

To prepare saturated solutions:

1. Add the solvent of choice, ~50-200 µl depending on need. Vortex well then let it sit at room temp. ~5 min. There should be undissolved EAM in the tube.
2. Microfuge 2 min. at maximum speed, room temp. The EAM is now ready to use. Keep at room temp. The solubility drops significantly when kept on ice.
3. Optional: Sometimes the CHCA particulate is floating on top of the solution. To minimize deposition of solid CHCA onto the spot, take the supernatant off the top of the CHCA and transfer it to another tube.

- When pipetting EAM solution, be careful not to touch the undissolved EAM at the bottom of the tube.
- Avoid having the EAM solution to spread outside the area of the spot. This will result in loss of sample outside the area of data collection.
- EAM compounds are light sensitive, and should be freshly prepared.
- Some volatile solvents evaporate even from closed containers (i.e., ACN), so it is best not to use premixed solvent beyond several days, at most.

Solvent Choice – Most common for ProteinChip protein/peptide analysis

- For most applications, the EAM molecules are prepared in an aqueous solution containing **50% acetonitrile (ACN)** and **0.5% trifluoroacetic acid**. All reagents are HPLC grade.
- As an alternative, a solvent containing **30% ACN, 15% isopropanol, 0.5% TFA** and **0.05% NP-40** (or Triton X-100) works well.
- For difficult proteins and peptides, especially the hydrophobic variety, try adding formic acid to the mix. Dissolve EAM in 150 µl of **50% ethanol** (100 proof). Microfuge, then transfer 90 µl of supernatant to a fresh tube. Add 10 µl of 100% **formic acid (10% final)**.

For glycoproteins, the addition of a small amount of detergent (0.02-0.1% OGP or NP-40) to the 50% acetonitrile and 0.5% trifluoroacetic acid solvent may be helpful.

EAM Selection – Detection of other Molecules

Many applications of the ProteinChip System require the detection of non-protein molecules, including nucleic acid, small compounds, and various polymers. Additionally, the 3 most commonly used EAM may not be optimally effective for a particular protein or peptide. In general, EAM choice (or Matrix, in the MALDI literature) is dependant on the chemical nature of the analyte. Listed here are a number of MALDI detection compounds that have been successfully used with the ProteinChip Technology (this list not comprehensive). Other compounds that work for MALDI will also work for SELDI. Experiment with different compounds according to your needs. Consider varying the solvent system as well.

Short name (MW, dal):	Full name:	Best for Detecting:
HPA (139.11 Da)	3-hydroxypicolinic acid	Oligonucleotides
DHBA, DHB, Gentisic acid (154.12 Da)	2,5-dihydroxybenzoic acid	small compounds, carbohydrates, peptides
THAP (168.15 Da)	2,4,6-trihydroxyacetophenone	organic compounds, oligonucleotides
Ferrulic acid (194.19 Da)	4-hydroxy-3-methoxycinnamic acid	Large proteins, peptides, amino acids
HABA (242.24 Da)	2-(4-hydroxyphenylazo)-benzoic acid	Synthetic polymers, proteins

Suggestions for optimization of analyte detection:

- Be willing to test more than one compound
- Consider other solvent systems, including MeOH or EtOH based solvents
- Check MALDI literature for recommendations of EAM, solvent, and additives
- Add sample to the spot before adding EAM. Don't premix as analyte might fall out of solution

If detection is proving difficult, consider these options:

- Check for "thin film" formation. This can occur with some polymers and compounds when added to the surface at high concentration. This is seen as a "shiny" surface before EAM addition. When this occurs, repeat the sample spotting with ~5-100-fold lower [analyte]
- In some cases (carbohydrates, some small molecules) the addition of small amounts of salt (1mM KCl or NaCl diluted 2-5-fold into sample) will aid in the ionization process, improving detection efficiency
- Add a small amount of BSA or other protein (0.05% diluted into sample) directly to the sample. Alternatively, coat the ProteinChip array with 100-200 fmol BSA (in H₂O). Dry the BSA, then add analyte followed by EAM
- Try dissolving the EAM in acetone. Then add 0.5 ul/spot of EAM/acetone, air dry. Then add analyte
- Frequently the detection of an analyte will be improved after binding to a ProteinChip array surface. Although this isn't always the case, it does occur in some cases, especially after capture by a protein.