ProteinChip® Energy Absorbing Molecules (EAM)

Instruction Manual

Catalog #C30-00001, #C30-00002, #C30-00003, #C30-00004

For technical support,
call your local Bio-Rad office, or
in the US, call **1-800-4BIORAD (1-800-424-6723)**.

BIO-RAD

Introduction

ProteinChip energy absorbing molecules (EAM) are an essential component of successful ProteinChip SELDI system experiments. EAM is a generic name for molecules that assist in desorption and ionization of the analyte. Known as “matrix” in traditional MALDI mass spectrometry, EAM are applied in organic solvent, solubilizing many proteins on the array surface. As the EAM dry, the proteins cocrystallize with the EAM. These crystals absorb the laser energy and generate the ionized proteins detected by the ProteinChip SELDI system.

Product Description

Bio-Rad offers three EAM for the detection of proteins and peptides. The general guidelines for choosing EAM are based on the molecular weight (MW) and chemical nature of the analyte, but there are no absolute rules.

ProteinChip CHCA EAM, 5 mg/vial

ProteinChip alpha-cyano-4-hydroxy cinnamic acid (CHCA) EAM (MW 189.2) are especially good for small molecules, 1–30 kD.

ProteinChip SPA EAM, 5 mg/vial

ProteinChip sinapinic acid (SPA) EAM (MW 224.2) are recommended for all larger proteins, but also work reasonably well for peptides. In general,

ProteinChip SPA EAM give better resolution and fewer multiply charged ions than ProteinChip EAM-1 EAM. For protein profiling using only one kind of EAM, ProteinChip SPA EAM are the molecules of choice.

ProteinChip EAM-1 EAM, 5 mg/vial

ProteinChip EAM-1 EAM are proprietary molecules (MW 231.21) and work well for proteins in the 10–50 kD mass range. ProteinChip EAM-1 EAM prove useful in allowing desorption and ionization of proteins that are difficult to detect, such as glycosylated proteins. It should be noted that ProteinChip EAM-1 EAM tend to generate multiply charged species, and peaks tend to be broader due to more adduct formation.

Preparing ProteinChip EAM in Solution

ProteinChip EAM in solution can be prepared in a number of different ways that may optimize the detection of the proteins in your sample. Table 1 to the right lists the steps for preparing EAM in solution using commonly used solvent systems. For a comprehensive list of solvent solution options, please see the ProteinChip SELDI System Applications Guide, Volume 1.

Table 1. Preparation of ProteinChip EAM in solution using commonly used solvent systems.

EAM	Solvent System	Preparation
ProteinChip CHCA	50% acetonitrile 49.75% HPLC grade H_2O 0.25% trifluoroacetic acid (TFA)	1. Add 200 µl solvent to vial (there should be undissolved CHCA in the bottom of the tube) 2. Mix 3. Centrifuge 4. Draw off supernatant and dilute with equal part solvent
ProteinChip SPA	50% acetonitrile 49.50% HPLC grade H_2O 0.50% TFA	1. Add 400 µl solvent to vial 2. Mix 3. Centrifuge
ProteinChip EAM-1	50% acetonitrile 49.50% HPLC grade H_2O 0.50% TFA	1. Add 200 µl solvent to vial (there should be undissolved EAM-1 in the bottom of the tube) 2. Mix 3. Centrifuge 4. Draw off supernatant and dilute with 2x volume of solvent

Solvent systems and ProteinChip EAM in solution are best prepared fresh every day. Acetonitrile volatilizes rapidly, and EAM in solution break down significantly within 24 hours at room temperature. Freezing EAM after dissolving them in solvent may preserve them for about a week; however, it is recommended to prepare fresh solvent and ProteinChip EAM in solution each day.

Preparing Saturated Solutions

1. Bio-Rad supplies ProteinChip EAM as 5 mg of dried powder in a tube. Tap the tube to ensure that contents fall to the bottom of the tube before reconstitution. Add 50–200 µl of the appropriate solvent depending on your needs. Vortex for approximately 5 minutes, then let sit at room temperature for approximately 5 minutes. There should be undissolved EAM remaining in the tube.
2. Microcentrifuge for 10 minutes at 10,000 rpm at room temperature to pellet any particulates. The ProteinChip EAM are now ready to use. Keep at room temperature. The solubility drops significantly when kept on ice.

Note: Occasionally CHCA particulate will float 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.

Applying EAM to Arrays

The method of ProteinChip EAM application to the array surface requires practice as it can significantly influence the quality of the data. A minute volume (0.3–1.0 µl) of ProteinChip EAM in organic solvent must be applied to each sample prior to analysis. It is recommended that ProteinChip EAM be applied to the spots after they have dried completely for the greatest spot-to-spot consistency.

If variable signal is seen across the spot when reading the arrays, it may be useful to observe the ProteinChip EAM in good light, or under a dissecting microscope. The EAM should be distributed fairly uniformly across the spot surface.

Note:

1. When pipetting ProteinChip EAM onto samples, be careful to avoid the undissolved EAM at the bottom of the tube.
2. Always use new, packaged pipet tips; never use tips that have been autoclaved or reused.

Storage

ProteinChip EAM are light-sensitive and should be stored away from the light.

Ordering Information

Catalog #	Description
C30-00001	**ProteinChip CHCA Energy Absorbing Molecules (EAM)**, 5 mg/vial, 20
C30-00002	**ProteinChip SPA Energy Absorbing Molecules (EAM)**, 5 mg/vial, 20
C30-00003	**ProteinChip EAM-1 Energy Absorbing Molecules (EAM)**, 5 mg/vial, 20
C30-00004	**ProteinChip Energy Absorbing Molecules (EAM) Kit**, includes 6 vials each CHCA, EAM-1, and SPA EAM

The SELDI process is covered by US patents 5,719,060, 6,225,047, 6,579,719, 6,818,411, and other issued patents and pending applications in the US and other jurisdictions.

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 4BIORAD*
Australia *02 9914 2800* ***Austria*** *01 877 89 01*
Belgium *09 385 55 11* ***Brazil*** *55 21 3237 9400*
Canada *905 712 2771* ***China*** *86 21 6426 0808*
Czech Republic *420 241 430 532*
Denmark *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 318 84 0*
Greece *30 210 777 4396*
Hong Kong *852 2789 3300* ***Hungary*** *36 1 455 8800*
India *91 124 4029300/5013478* ***Israel*** *03 963 6050*
Italy *39 02 216091* ***Japan*** *03 5811 6270*
Korea *82 2 3473 4460* ***Mexico*** *55 5200 05 20*
The Netherlands *0318 540666*
New Zealand *64 9415 2280* ***Norway*** *23 38 41 30*
Poland *48 22 331 99 99* ***Portugal*** *351 21 472 7700*
Russia *7 095 721 14 04* ***Singapore*** *65 6415 3188*
South Africa *27 0861 246 723* ***Spain*** *34 91 590 5200*
Sweden *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189/2578 7241*
United Kingdom *020 8328 2000*

10008246 US/EG Rev E *06-0680 1006 Sig 1205*