

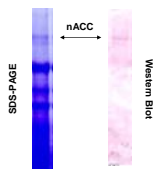
## Abstract

Acetyl-CoA carboxylase (ACC) is a highly validated drug target with relevance to obesity, diabetes and metabolic disorder. Unfortunately, difficulties in generating and isolating active enzymatic preparations, as well as problems developing practical screening techniques, have impeded progress in the development of viable drug candidates. To date, identification of new lead compounds showing inhibitory activity have been limited to low-throughput radiometric (<sup>14</sup>C) incorporation assays. These technologies are time-consuming, expensive and generate significant amounts of radioactive waste precluding large scale screening efforts. Using an ACC enriched fraction from rat liver, we have been able to develop an effective high-throughput screening methodology utilizing a proprietary high-throughput mass spectrometry system as a detection tool. Mass spectrometry allows for the direct, quantitative detection of native, label-free enzymatic substrates and products (i.e. acetyl-CoA/malonyl-CoA) facilitating functional assay screening of previously intractable targets.

## Native Acetyl-CoA Carboxylase Isolated from Fast-Fed Rat Liver

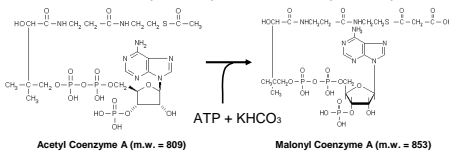
(BlueSky Biotech, Inc. Cat #: 1041-005)

The enzyme is a multi-step extraction/enrichment, isolated from the livers of Fast-Fed Rats. This product contains background proteins that co-purify with nACC, but do not negatively affect the activity.



## Acetyl-Coenzyme A Carboxylase Assay

- Acetyl-CoA carboxylase (ACC) activity is the first and rate-limiting step in the biosynthesis of long-chain fatty acids
- ACC is an important target for the development of therapeutics against obesity, diabetes and metabolic disorder
- ACC converts Acetyl-Coenzyme A into Malonyl-Coenzyme A



## Materials

Acetyl-CoA Carboxylase	BlueSky Biotech*
Acetyl Coenzyme A	Sigma-Aldrich
Malonyl Coenzyme A	Sigma-Aldrich

\*ACC enzyme was purified from fast-fed rat livers by BlueSky Biotech, Inc.

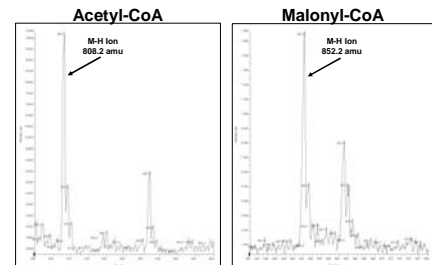
## Assay Buffer\*

50 mM HEPES	2 mM MgCl <sub>2</sub>
2 mM potassium citrate	0.75 mg/ml BSA
2 mM DTT	4 mM ATP
12.5 mM KHCO <sub>3</sub>	

\*ACC assay conditions based on previously published <sup>14</sup>C-incorporation assay protocol: Harwood, H.J., Jr. et al. *J. Biol. Chem.* (2003), 278 (39): 37099-37111

## MS Method Development

MS conditions were optimized using an Agilent 1100 HPLC system interfaced to a Sciex API-4000 triple quadrupole mass spectrometer

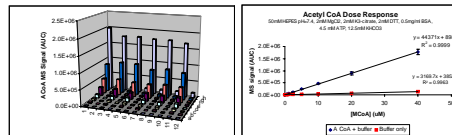


## Assay Development

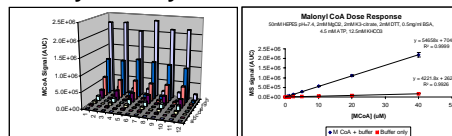
- The assay was ported onto BioTrove's proprietary RapidFire™ high-throughput mass spectrometry system
- Throughput = 1 sample per 7 seconds
- Limits of quantitation for ACoA and MCoA were determined by analyzing serial dilutions of the compounds in a 96-well plate
- Sample in assay buffer and assay buffer only were alternated in each column to investigate system carryover
- The Coenzyme A derivatives were analyzed by ESI-MS/MS in negative ion mode

• ACoA MRM	Q1: 808.5 (M-H ion)	Q3: 408.1
• MCoA MRM	Q1: 852.6 (M-H ion)	Q3: 408.1

## Acetyl-Coenzyme A Serial Dilution Data

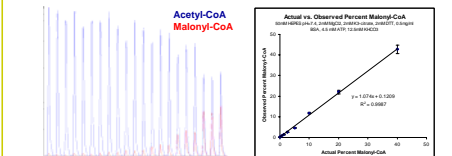


## Malonyl-Coenzyme A Serial Dilution Data



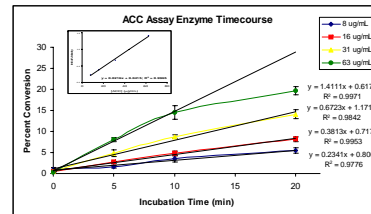
## ACoA/MCoA Simultaneous MS Detection

The two analytes were combined such that the total concentration of analyte was maintained at 20 µM. The actual percent conversion was then plotted against the experimentally observed percent conversion.

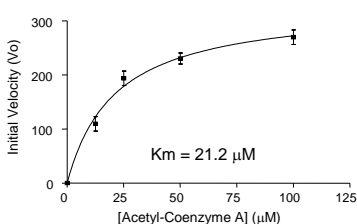


## Enzyme Linearity

- 20 µM Acetyl CoA, room temp incubation
- Enzyme linearity observed between 8 µg/mL and 63 µg/mL



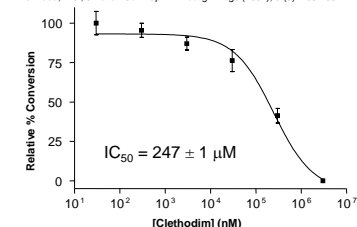
## Km Determination



## Clethodim IC<sub>50</sub> Determination

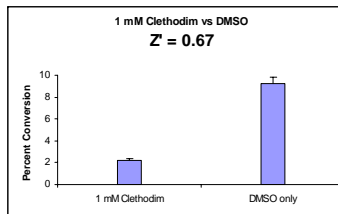
It has been reported in the literature that some cyclohexanone compounds are inhibitors of the ACC enzyme. Clethodim was purchased from Sigma (St Louis, MO) and its activity against ACC was determined.

\* Harwood, H.J., Jr. et al. *Curr. Opin. Investig. Drugs* (2004), 5 (3): 283-289



## Z' Score Determination

$$Z' = 1 - (((\sigma_{high} + \sigma_{low})) / (\Delta v_{high} - \Delta v_{low}))$$



## Conclusions

- We developed an assay that can be used for the discovery of inhibitors against ACC using high throughput mass spectrometry at a sustained throughput of 7 seconds per sample
- We determined enzyme kinetics for the assay including a Km and linear concentration ranges
- We observed good statistical parameters (Z' = 0.67) using a previously identified ACC inhibitor

## BlueSky Biotech, Inc.

The mission of Blue Sky Biotech is to relieve scientists from the onerous and labor-intensive tasks that take them away from key experimentation, novel theory exploration, and other optimal uses of their time. Blue Sky is synonymous with productivity in the Early Discovery Biology processes known as genetic cloning, recombinant protein expression, and laboratory-scale fermentation (scale-up bioprocessing). We are motivated by our ability to impact the overall efficiency of the drug discovery process, thus contributing to a longer, healthier lifespan for human beings. 1(800)383-7795

## BioTrove's RapidFire™ Lead Discovery Service

BioTrove scientists collaborate with researchers to design mass spectrometry-based high-throughput screening assays. These assays enable the identification of lead compounds against drug targets that may have previously been thought to be too expensive or technically impossible to screen. Over the past few years in multiple campaigns, BioTrove scientists have developed dozens of assays and leveraged mass spectrometry to screen over 5 million compounds. 1(781)721-3605