LC Laboratories®

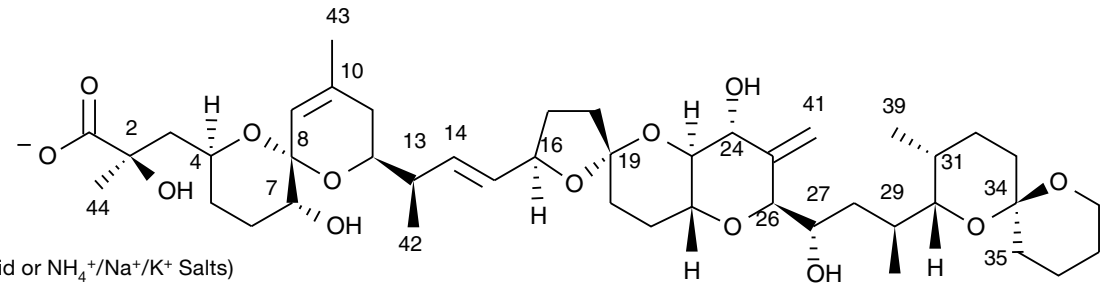
Technical Note #18

Revised and Updated October 25, 2016

High Purity, Low Cost Okadaic Acid: A Potent Inhibitor of Serine/Threonine-Specific Protein Phosphatases

Adapted and updated from previous LC Labs publications relating to okadaic acid:
New Product Bulletin (December, 1990), *New Product Bulletin.* (November, 1991), and
The Messenger. **Vol. 1, No. 2** (August, 1992) and **Vol. 3, No.1** (July, 1994)

Figure 1: Okadaic Acid



(Free Acid or NH_4^+/Na^+/K^+ Salts)

1990: The First High Purity, Low Cost Okadaic Acid

In late 1990 LC Laboratories became the first company to introduce high-purity, low-cost **okadaic acid** ("**OA**") to the worldwide bioresearch community, at prices 80% below those of the nearest competitor.

Since that time, at least 500 labs worldwide, and probably hundreds more, have used OA from LC Labs (either directly from us or from our numerous distributors, many of whom resell our products under their own labels).

OA (Fig. 1) is the best known of a small family of diarrhetic shellfish toxins. It is produced by dinoflagellates[1, 2] and can be recovered from several other types of marine organisms[3,4] whose diets presumably include the OA-producing dinoflagellates. Related toxins include dinophysistoxin-1, which has one more methyl group than OA[5] and acanthifolicin, which is an episulfide derivative of OA[6].

OA is an important tool for biological research. It is a potent inhibitor of serine/threonine protein phosphatases present in mammalian cells including, PP1, PP2A, PP4, PP5, and PP6. It is also able to inhibit, though less potently, other phosphatases such as PP2B and PP7 (see Table 1 on page 2 for IC_{50} values against many protein phosphatases, for various substrates).

However, OA appears to have no effect on PPM (Mg- or Mn-dependent protein phosphatase) family members or tyrosine phosphatases[7-15]. OA appears to exert its biological effects by causing a net increase in the prevailing levels of phosphorylated proteins, an effect which is equivalent in many ways to activating various serine/threonine-specific protein kinases (Fig. 2 on page 3).

Both OA and calyculin A are commonly used *in vitro* and *in vivo* to study the actions of protein phosphatases.

OA is a very potent, non-phorbol ester-type tumor promoter on mouse skin (thus mimicking the widely-used tumor promoter phorbol 12-myristate 13-acetate) and rat glandular stomach[16,17]. It stimulates glucose transport in adipocytes[14], increases calcium currents in isolated myocytes[15], inhibits translation in reticulocyte lysates[18], and causes both relaxation[19] and contraction[20] of isolated aortas. Given the ubiquity of kinase/phosphatase systems in eukaryotic cells, many other specific effects of OA are likely to be found as research with this compound continues.

Okadaic Acid Stability; Salt Forms

The free acid form of OA suffers from a subtle but very serious stability problem, which LC Laboratories solved shortly after we introduced this product.

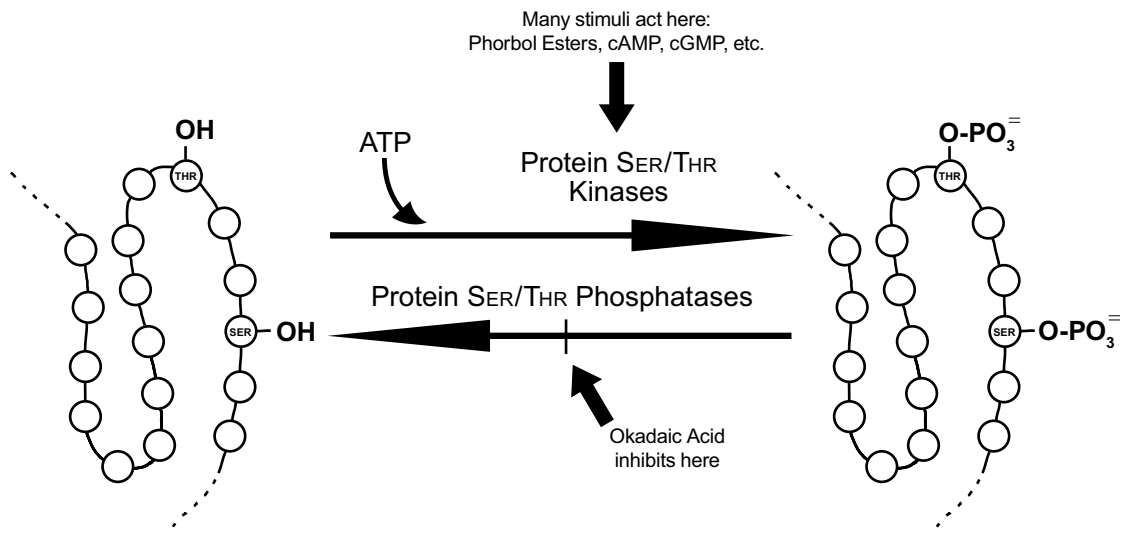
As a final purification step, OA can be crystallized to >99% purity and stored as such *in bulk* for long periods of time at -20 ºC without developing impurities.

However, when OA is dissolved in any of a wide range of common solvents, distributed into ampules and dried into a thin film, or when it is dissolved and stored in DMF, it can

Table 1: Phosphatase Inhibition Profiles for Okadaic Acid – IC_{50} (nM)

Phosphatase	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.	Substrate	Ref.
	Phosphohistone		**Phosphorylated myosin light-chain (PMLC)**		**Phosphorylase α**		**LC20**		**p-Nitrophenyl phosphate**		**Myelin basic protein (MBP)**		**poly (Glu:Tyr) or reduced carboxamidomethylated and maleylated lysozyme**		**Inositol trisphosphate**	
PP1	15-50	[7]	315	[8]	272	[8]	330	[15]								
"	42	[10]	500	[15]	19	[11]										
"					3.4	[13]										
"					60	[15]										
PP2A	0.1-0.3	[7]	1.2	[8]	1.6	[8]	0.5-1.0	[15]	0.1	[12]	10	[12]				
"	0.51	[10]	0.5-1.0	[15]	0.2	[11]										
"			100	[14]	0.5-1.0	[15]										
"					0.07	[13]										
PP2B (calcineurin)	~4,000	[7]	4,530	[8]					3,600	[8]						
"			10,000	[14]												
PP2C			>10,000	[8]												
"			>10,000	[14]												
PP4	0.1	[7]														
PP5	3.5	[7]														
PP6									0.1	[12]	2	[12]				
PP7	>1,000	[7]							>1,000	[9]						
"	>1,000	[9]														
polycation-modulated phosphatase (PCM)			205	[8]	72	[8]										
Protein tyrosine phosphatase													>10,000	[13]		
"													>10,000	[8]		
Inositol 1,4,5-trisphosphate phosphatase															>50,000	[8]
Acid phosphatases									>10,000	[8]						
"									>10,000	[15]						
Alkaline phosphatases									>10,000	[15]						
"									>10,000	[8]						

Figure 2: The Protein Kinase/Phosphotase Cycle



quickly begin to develop 20-50% of impurities. OA's close relative, 35-methylokadaic acid, suffers the same problem. The smaller the quantity of OA that is spread out as a thin film in an ampule, the worse the stability problem.

Many okadaic acid suppliers are not aware of, or simply ignore, this major stability problem. Fig. 3 on page 4 compares TLC analyses of a recent, typical lot of LC Laboratories' OA free acid with OA samples obtained at various times from three other well-known vendors.

Large amounts of impurities are evident in the samples from the other vendors. It is therefore not surprising that some researchers have told us that our OA is more potent than that obtained from other sources.

LC Labs took two approaches to solving the OA instability problem.

First, we tested and introduced three salt forms of OA (sodium, potassium, and ammonium) that have modestly improved stability, both in the unopened vial and after dissolution and storage in DMSO or ethanol. However, as Figure 3 shows, these three salt forms can also suffer the same general instability problem as the free acid. The salt forms can be used, rather than the free acid, in experiments in which the K, Na or NH_4 cations will not interfere. Since OA is always ionized to a salt form in physiological media, the biological activity of the salt forms is the same as that of the free acid, after the slight differences in molecular weight have been taken into account.

Second, we found methods to greatly improve the storage stability of microgram quantities of all four OA forms. We are thus able to routinely offer our four OA products with purity guaranteed to be >98%. ***We believe our OA products are absolutely the purest and most stable material available from any source worldwide***.

In addition to the thin-film stability problem, the surfaces of even Type I Borosilicate glass ampules also introduce additional, somewhat less serious impurity problems. LC Labs has learned to solve these other problems by developing and using a non-standard surface treatment for our ampules.

Important Note: **Although OA and its salts are somewhat soluble in water, solid OA and its salts must first be dissolved in an organic solvent, such as DMSO or ethanol, before introducing water into the solution. Attempts to dissolve OA or its salts directly in water or buffers generally fail to dissolve all of the solid material**.

Pricing

O-2220 Okadaic Acid, Free Acid, >98%
O-6410 Okadaic Acid, Ammonium Salt, >98%
O-7519 Okadaic Acid, Potassium Salt, >98%
O-5857 Okadaic Acid, Sodium Salt, >98%

Size	US$	€	£	¥
50 µg	**48**	40	35	5,300
100 µg	**85**	71	62	9,400
300 µg	**228**	190	167	25,300
1 mg	**595**	497	437	66,000

NOTE: Euro, Pound and Yen prices are revised regularly.
Please visit www.LCLabs.com for our current prices.

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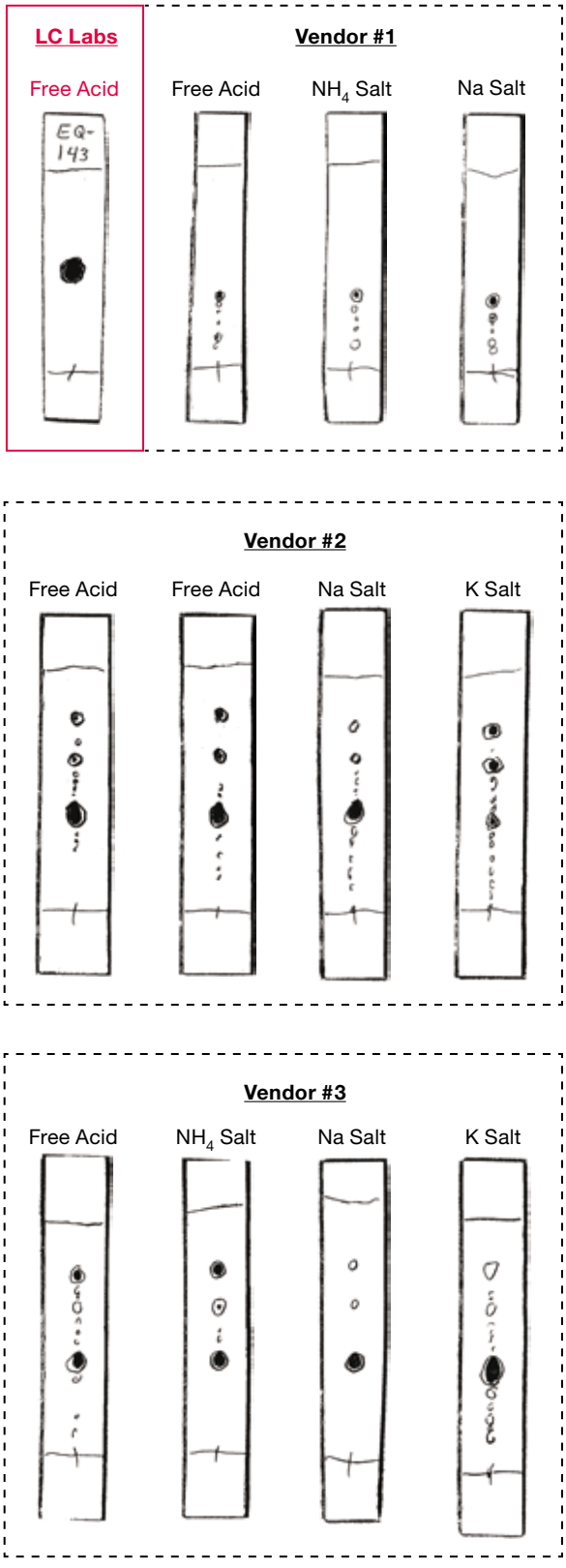
Figure 3:
Purity of OA Products from LC Labs and Three Other Vendors



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